SOME EFFECTS OF AN EXCESS INTAKE OF CARBOHYDRATE ON MAN AND RATS.

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C O N T E N T S

CHAPTER I.

INTRODUCTION

In spite of tremendous advances in the knowledge of nutrition in recent years the problems of the human diet are still centred around the two extremes of overnutrition and undernutrition. There are many people in the world whose health is impaired by a food intake which is either overabundant or else inadequate to meet their nutritional needs. The present investigation deals with certain aspects of overnutrition with particular reference to an excess intake of carbohydrate.

If the intake of energy-yielding nutrients is greater than the expenditure of energy then storage of metabolites must result in fulfillment of the laws of thermodynamics. The fact that the body weight of many adults remains fairly constant over long periods indicates their ability to maintain an energy balance, though day-to-day varia-:tions in body weight frequently occur and chiefly reflect changes in water content. For some people however, there is a failure to maintain this energy balance; their food intake is too great in relation to their energy expen-:diture, and an increase in weight is the result. Though many aspects of overweight have been studied in recent years there have been very few experiments planned specifically to study the effects of overeating.

Excess dietary carbohydrate may be stored as glycogen mainly within the cells of liver, muscle, adipose tissue

and blood. The total amount of glycogen in the human body is usually considered to be small though estimates have been based largely on the levels found in the tissues of laboratory animals. If glycogen storage is limited, a net conversion of carbohydrate into fat would be ex-:pected with a carbohydrate intake in excess of energy requirements. The conversion of dietary carbohydrate to body fat in animals was demonstrated by Lawes and Gilbert (1853) and is a well established fact.

The production of energy in animals depends ulti-:mately on the oxidation of foodstuffs by atmospheric O_2 with the consequent production of CO_2, H_2O and urinary N. Hence by measuring the O_2 utilisation and output of CO_2 and urinary N it is possible to calculate the amounts of protein, carbohydrate and fat metabolised in a given period. The early investigators observed that the ratio of the volume of CO_2 expired divided by the volume of O_2 used varied depending on the type of foodstuff being oxidised, since the proportions of carbon and oxygen differ in the 3 proximate principles, protein, fat and carbohydrate. The term Respiratory Quotient (RQ) was introduced for this ratio.

The theoretical RQ for the complete oxidation of glucose is 1.0 and of tripalmitin is 0.703., but the actual gaseous exchange in the metabolism of carbo-:hydrates and fats will depend on their particular content of monosaccharides and fatty acids respectively. For

proteins it is more difficult to calculate a theoretical RQ because of their incomplete oxidation but determinations based on chemical analyses of food proteins and of urine and faeces yield values of 0.79 - 0.82.

The conversion of carbohydrate into fat has a high RQ since carbohydrate molecules contain more oxygen relative to carbon and hydrogen than do molecules of fatty acids. RQ's well above 1.0 have been recorded in animals which are laying down fat and it should be possible to demonstrate this conversion in man by measuring RQ's after a dietary excess of carbohydrate. This thesis records measurements of RQ's and changes in body weight under such conditions.

Three sets of experiments were planned to study the effects of an excess intake of carbohydrate. In the first 2 sets of experiments the responses to overeating of human subjects were measured. In the third set, rats were used and their response to a dietary excess of carbohydrate was studied by measurement of the RQ and of changes in tissue glycogen.

The first set of experiments on the human subjects was of short duration and included 3 series of experiments:-

Series 1: Nine healthy subjects were each given 4 meals rich in carbohydrate and their response was studied for 14 h.

Series 2: The experiments were similar to those of Series 1 but the time was increased to 24h. and 5 meals larger in

size were taken by 8 men. The full water balance was recorded.

Series 3: The effects of a standard lunch were studied with 2 subjects during 8 days.

The second set of experiments were of longer duration and were performed in more detail on 2 human subjects. Two overweight young women were studied while in hospital for 23 days, during which they were given a control diet for 9 days, an excess intake for 9 days and a very small diet for the remaining 5 days. For the whole period the water balance was recorded, the diet, urine and faeces were analysed and measurements were made of O_2 consumption and CO_2 output during representative activities throughout the day and night.

A preliminary report of some of these experiments has already been published (Passmore & Swindells, 1962).

CHAPTER. II.

A REVIEW OF SOME OF THE RELEVANT LITERATURE.

A. FORCED-FEEDING IN MAN.

The literature reviewed in this section includes only those experiments in which the effects of an excess intake of food superimposed on a diet which is already adequate, have been studied using healthy human subjects. In order to understand the causes of obesity it seems im-:portant to study human subjects while they are actually gaining weight. However, the investigations in this category are very few in number.

In Table 1 are summarised 3 experiments which were carried out to determine whether thin people gain weight rapidly when given an excess of food. Table 1 shows the length in days of the control and experimental periods and gives the caloric intakes and changes in body weight and composition.

Gulick (1922) experimenting on himself found his weight of 62 kg. (height 181 cm.) remained constant with an intake of 2740 kcal. In 5 periods varying from 7-73 days in length during 15 months, he ate a diet rich in carbohydrate supplying 3380-4120 kcal. Records of activity were kept for part of the experiment and a pedometer was used for 4 weeks but the diet was not analysed. Gulick gained 13 kg in 14 months which re-:presented 83 g./day during the 5 periods of the ex-:periment. The N balance was recorded only for the final

period when the N retention was equivalent to 12 g. protein per day. As the caloric expenditure was not measured it is not possible to determine the size of the excess intake.

Wiley & Newburgh (1931 a,b) conducted a shorter experiment on a man aged 28 weighing 57.5 kg. (height 180 cm.) After a control period of 18 days on a net caloric intake of 2920 kcal. he was given a diet supplying 4760 kcal./day for 15 days; in the latter diet more than 60% of the calories were supplied as fat. The diet, urine and faeces were analysed and the calculation of the metabolic mixture was based on the carbohydrate content of the diet, the urinary N and the insensible weight loss (Newburgh, Wiley & Lashmet, 1931). The subject gained 294g./day while receiving 1680 kcal. in excess of his energy expenditure. This represents 5.7 kcal./g. gain in weight.

Passmore, Meiklejohn, Dewar & Thow (1955 a,b) overfed 3 thin men for 10-14 days with 1300-1630 kcal. in excess of their needs. Energy expenditure was measured and records of activity were accurately kept. The respective heights and weights of the 3 subjects were 61.8 kg., 180 cm.; 59.2 kg., 180 cm.; 57.0 kg., 172 cm. and their gains in weight were 150-187 g./day equivalent to 8.3 to 9.3 kcal./g. These men gained very little body water during the over feeding period in contrast to Wiley & Newburgh's results where 36% of the gain in weight was

calculated to be water.

In addition to the 3 experiments summarised in Table 1, the effects of an increased food intake on N balance have been studied in a number of cases. Pettenkofer & Voit (1866) using their newly-constructed respiration chamber measured the metabolism of a man on a protein-:rich diet (42.6gN/day) and demonstrated a mean positive N balance of 10.2g. in 2 experiments each lasting 24 h. In 1869 Voit reported large positive N balances when high-:calorie diets rich in both carbohydrate and protein were fed.

In Table 2, are given details of some longer ex-:periments of this kind. The table indicates the length in days of the control and experimental periods and the daily intakes in kcal. and g.N. The changes in body weight and retention of protein are also given. Krug (1894; quoted by Luethje, 1902) brought himself into N equilibrium after 6 days on a diet supplying 2550 kcal. When the intake was increased by 1710 kcal. by the addition of carbohydrate and fat for 15 days he retained N equivalent to 9g. protein/day. Bornstein (1898) added casein to a maintenance diet and retained 16g.N in 14 days. Dapper (1902; quoted by Magnus-Levy, 1907) also reported a large retention of N when the diet was increased from 2930 to 3400 kcal. by the additions of carbohydrate and protein.

Luethje (1902) was able to study the N balance and

changes in body weight of a woman aged 40, for a long per-:iod. After recovering from a neurosis she was in good health and her N equilibrium was studied for 7 weeks. In these experiments Luethje varied the caloric and pro-:tein intakes and used only a few foods which were easy to measure and analyse. The N retentions which he reported are very large but the daily intakes of calories and pro-:tein were also large except for the second period of the experiment. However, figures for N retention are subject to considerable error since they are obtained by difference from the total intake and output; no allowance was made for cutaneous losses in Luethje's experiments. With this subject an excess intake (4060 kcal.) was followed by a token diet for 9 days, including 4 days of complete starvation. When the excess intake was resumed the daily N retention was much greater than the negative balance during the starvation period. Period 4 has been omitted from the table as very large menstrual losses were re-:corded for 7 days.

In a later series of papers (Cuthbertson, McCutcheon & Munro, 1937; Cuthbertson & Munro, 1937; Cuthbertson, McGirr & Munro, 1937), the effects of overeating on N balance were discussed in more detail. In the first 2 papers of this series experiments were described in which Il. milk or its equivalent (as beef + lactose + butter; or soyabean + lactose + butter) was superimposed on an adequate basal diet. Mean daily N retentions representing

22-54% of the N intake were recorded in these experiments. Also in the second paper are reported experiments in which the sparing effect on protein metabolism of added carbo-:hydrate and fat was measured. The additions provided 700-780 kcal. except in the last experiment mentioned in Table 2 where a supplement of 1560 kcal. as glucose was given with the maximum N retention of the series. There were large daily changes in body weight in some of these experiments. Wilson (1931) working in the same depart-:ment had carried out similar experiments to those of Cuthbertson et al. (1937) but had determined the retention of N and S when beef alone was added to a basal diet. In the third paper of the series Cuthbertson et al. (1937) reported the effects on the N equillbrium of an excess in-:take of carbohydrate when a measured amount of work (on a bicycle ergometer) was performed either before or after a meal. The effects were observed first with a basal diet and later with added carbohydrate, either equivalent to the work performed or given in excess amounts over a long period. There was a larger rise in the excretion of N and S when the work was performed after a meal; when the work experiment took place during the extended carbohy-:drate-feeding the rise in N excretion due to work follow-:ing a meal was greater than the sparing effect of the added carbohydrate.

As observed by Cuthbertson & Munro (1937) forced-:feeding experiments with human subjects are usually

limited by lack of appetite. A further difficulty arises from the need to measure the energy expenditure during the overfeeding period in order to determine accurately the size of the calorie excess. Recently Ashworth, Creedy, Hunt,Mahon & Newlands (1962) reported that a nightly food supplement of 1000or 2000kcal. had no effect on the voluntary food intake of 5 subjects even after 20-36 days. This was in contrast to a similar experiment by Fryer (1958) in which 12 students received a 1000 kcal. supplement nightly for 8 weeks and voluntarily reduced their food intake by 50%. The mean gain in weight for this group was 56g./day. Both of these experiments would have been far more valuable if accurate measurements had been made of the food intake and the energy expenditure.

The errors which can be introduced by failing to measure the energy intake or expenditure are indicated by the findings of Taggart (1962) and of Newburgh, Johnston, Lashmet & Sheldon (1937). Taggart weighed her own diet daily for 80 days and found that her caloric intake varied from 1570 - 3490 kcal. To her surprise she also discovered that her mean intake on Sundays was 850 kcal, greater than on mid-week days. Newburgh et al. demonstrated a similar variation in energy expenditure. The energy output of a highly trained subject during 11, 24h. periods in a metabolic chamber varied from 1730 to 2240 kcal.and the subject was unable to predict the level. When Keys, Anderson & Brozek (1955) persuaded 20 schizo-

:phrenic patients to overeat for 6 months the weight gains of the subjects varied from 2.5 to 22.3 kg while their activity remained "constant". As no details of the diet are given it is not possible to estimate the extent of the calorie excess.

In the experiments summarised in Tables 1. and 2. energy expenditure was measured directly by Passmore et al. (1955 a,b) and indirectly by Wiley & Newburgh (1931 a,b). Leuthje (1902) and Krug (1894) calculated the excess intake by assuming a figure for the caloric re-:quirement related to the body weight in each period. From the calorie excess and the N retention they cal-:culated the amounts of fat and protein stored. In the experiments of Gulick (1922) and of Cuthbertson et al. (1937) attempts were made to keep the daily energy ex-:penditure as constant as possible in each period.

Table 1.

The length in days of the control and experimental periods, the energy intake and the change in body weight and composition of 5 thin men who were overfed.

REFERENCE	CONTROL PERIOD		EXPERIMENTAL PERIOD			BODY WEIGHT			CHANGE IN BODY COMPOSITION		
	Days	Intake kcal./day	Days	Intake kcal./day	Output kcal./day	Initial kg.	Gain g./day	Gain kcal/g.	Protein g./day	Fat g./day	Water g./day
Gulick (1922)	77	2740	16	3480		61.6	141				
			18	3810		63.9	129				
			50	3380		70.1	30				
			73	3550		71.2	24				
			7	4120		74.1	91		12		
Wiley & Newburgh (1931 a,b)	18	2920	15	4760	3080	57.5	294	5.7	12	166	106
Passmore, Meiklejohn, Dewar & Thow (1955 a,b)	4	2100	10	3730	2430	61.8	150	8.7	19	129	2
	4	2100	14	3970	2340	59.2	175	9.3	22	163	-10
	4	2140	14	3920	2360	57.0	187	8.3	14	160	13

Table 2.

The length in days of the control and experimental periods, the energy and nitrogen intakes, the changes in body weight and the protein retention during overfeeding.

REFERENCE	CONTROL PERIOD			EXPERIMENT			BODY WEIGHT		Protein retention g./day	Additions to basal diet
		INTAKE			INTAKE					
	Days	kcal./day	N g./day	Days	kcal./day	N g./day	Initial kg.	Gain g./day		
Krug (1894)	6	2550	20	15	4260	20	72	206	9	Fat & carbohydrate
Bornstein (1898)	4		14.9	14		21.9	70	43	7	Protein
Dapper (1902)	-	-	-	6	2930	20.3	90		14	
				12	3230	20.1			21	Starch
				9	3400	24.6			16	Starch & Protein
Luethje (1902)	-	-	-	10	4060	49.1	60.9	132	92	
				9	300	2.6	62.2	-668	-49	
				8	3770	46.2	56.2	453	116	
				13	3380	28.8	62.4	137	41	
Cuthbertson McCutcheon & Munro (1937)	14	3300	14.9	8	3980	20.6	86.2	90	12	1 l. milk
	14	3300	14.9	8	3980	20.8	86.5	69	9	beef and lactose and butter equivalent to 1 l. milk. †
	14	2850	12.0	8	3530	17.8	61.4	50	11	
	14	2850	12.0	8	3530		60.5	88	12	
	8	2850	12.1	15	3550 †	17.9	61.2	83	6	
Cuthbertson & Munro (1937)	9	3400	15.1	5*	3600	9.0	86.9	103	16	Beef and lactose and butter (beef only added*)
				5	4060	9.0	87.3			
	7	3200	10.6	3	3980	10.6	87.1	93	7	Carbohydrate
	3	3200	10.6	3	3900	10.6	87.4	(143)	2	Fat
	6	2890	10.6	6	3590	10.6	63.5	0	7	Fat
	6	2890	10.6	5	3670	10.6	63.3	200	11	Carbohydrate
					4450	10.6	64.7	233	21	

B. HUMAN GLYCOGEN RESERVES.

A study of the literature was made to determine the present state of knowledge of human glycogen reserves, since the level of these reserves is important in a dis-:cussion of the fate of an excess intake of carbohydrate.

Soskin and Levine (1952) stated that a hypothetical 70 kg. man would contain 370 g. of total body carbohy-:drate, calculated in the following manner:-

	Weight kg.	Glycogen %	Total carbohydrate g.
Muscle	35	0.70	245
Liver	1.8	6.00	108
			353
Sugar in blood and extracellular fluids			17
			370

This calculation was originally reported by Soskin (1943) when trying to estimate the carbohydrate required by a diabetic comatose person during the first 24 h. of treat-:ment with insulin. This figure of 370 g. is often quoted as the level of carbohydrate storage in man.

Direct determinations of the glycogen content of human muscle and liver have been made in a few instances. Autopsy measurements are of limited value since glycogen is a very labile tissue constituent and some investigators have ignored the large post-mortem losses when reporting their results. MacIntyre, Pedersen & Maddock (1941) have reviewed the early autopsy measurements of liver glycogen.

Even biopsy samples taken during an operation will pro-:bably have a reduced glycogen content and the level may be influenced by the particular anaesthetic in use. (MacIntyre et al., 1941). However, the taking of small samples by needle biopsy has now become an established clinical technique and is suitable for glycogen which can be estimated by microchemical methods. In Tables 3 and 4 are given the glycogen content of human muscle and liver, as reported in the literature. A few autopsy results have been included for comparison with the biopsy values.

Only Hildes et al. (1949) have measured muscle and liver glycogen in samples obtained by needle biopsy from the same subjects. The difference they found between the glycogen content of the gastrocnemius and pectoralis major muscles, indicates the difficulty in using such re-:sults as the basis for calculating the glycogen reserves of the whole body. However samples taken in standard conditions both before and after a particular treatment can indicate changes in glycogen levels. Bondy et al. (1949) observed increases in liver glycogen of 1.5 and 2.3% when samples were taken after breakfast instead of in the fasting state. Holmes & Trowel (1948) found that liver glycogen rose from 2.2 to 2.8% when 5 healthy sub-:jects were given 50g. glucose intravenously. Cramer (1888) reported much higher muscle glycogen levels than are usually found in autopsy investigations but the samples

were from new-born infants. In most animals the muscle glycogen at birth is at least twice the adult level though it falls rapidly during the first 3 days of life. (Shelley, 1961). Glycogen Storage Diseases. Some of the biopsy measurements reported in Tables 3 and 4 were made in order to indicate normal glycogen reserves for comparison with levels found in glycogenoses. This term covers a group of rare diseases in which large amounts of glycogen accumulate in the tissues due in many cases to a lack of one or more of the enzymes essential for glycogen metabolism. The glycogenoses are classified in the following manner (Cori, 1954; Illingworth, 1961) depend-:ing on the particular enzyme deficiency:-

Type 1: Glucose -6- phosphatase deficiency.

Type 2: Generalised glycogenosis.

Type 3: Limit dextrinosis (lack of amylo-1, 6-glucosidase, the debranching enzyme).

Type 4: Glycogenosis (probably lack of amylo-1, 4→1, 6 transglucosidase, the glycogen branching enzyme)

Type 5: Glycogenosis with absence of muscle phosphorylase.

Type 6: Glycogenosis of liver.

Attempts to demonstrate an enzyme deficiency in Type 2 have been unsuccessful (Hauk, Illingworth, Brown & Cori, 1959; Hug, 1961). Since normal glycogen has a very branched molecular structure, the glycogen which ac-:cumulates in Types 3 and 4 is abnormal in structure.

The fact that phosphorylase is active in glycogen break-:down but not in its synthesis was confirmed by the dis-:covery of Type 5 glycogen storage disease. (Mommaerts et al., 1959).

Table 3.

The glycogen content (in g./100g.) of human muscle, as reported in the literature.

	REFERENCE	NO. OF SUBJECTS	GLYCOGEN CONTENT		COMMENT
			RANGE	MEAN	
Samples taken by needle biopsy.	Hildes, Sherlock, & Walshe (1949)	15	1.19 - 3.89	2.20	Pectoralis major
		11	0.78 - 2.19	1.30	Gastrocnemius
	Mommaerts, Illing-:worth, Pearson, Guillory, & Seray-:darian (1959)	1		0.93	Control for study of glycogen storage disease in muscle.
Samples taken during an operation.	Illing-:worth & Cori (1952)	3	0.56 - 0.96	0.69	Operation for breast cancer.
	Polglase, Smith & Tyler (1952)	2	0.5, 0.7	0.6	Muscular disease without glycogen storage.
Autopsy Samples	Cramer (1888)	3	0.87 - 1.85	1.31	Newborn infants
	Yater, Osterberg & Hefke (1930)	21	0.08 - 0.89	0.41	Cardiac muscle only.
	Illing-:worth & Cori (1952)	1		0.1	Glycogen storage disease of liver; muscles unaffected.
	Polglase et al. (1952)	1		0.06	Infant.

Table 4.

The glycogen content (in g./100 g.) of human livers, as reported in the literature.

	REFERENCE	NO. OF SUBJECTS	GLYCOGEN CONTENT		COMMENT
			RANGE	MEAN	
Samples taken by needle biopsy after an overnight fast.	Haex (1944)	15	6.3 - 9.7	6.5	Peptic ulcer
		5	2.1 - 4.4	3.0	Hyperthyroid
	Holmes & Trowel (1948)	5	1.0 - 3.7	2.2	Healthy East Africans
	Bondy, Sheldon & Evans (1949)	3	2.8 - 4.7	3.6	Acute illness not involving liver.
	Hildes et al (1949)	19	1.0 - 4.1	2.2	Convalescent hospital patients.
	Bergström, Findnor & Hultman (1961)	3	2.3 - 4.4	3.6	Chronic arthritis
		6	4.0 - 6.1	5.2	Healthy subjects
Samples taken during an operat-:ion	MacIntyre, Pedersen, & Maddock (1941)	10	1.1 - 6.3	3.2	Spinal anaesthesia
	Young, Abels & Homberger (1948)	14	0.4 - 4.2	2.0	Benign gastric lesions
	Polglase et al. (1952)	1		2.1	Peptic ulcer
	Sokal & Gerszi (1959)	37	0.3 - 6.0	2.2	Carcinoma patients mainly.

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Table 4 (Contd.)

Autopsy samples.	Cramer (1888)	3	1.0 - 2.2	1.5	Newborn infants.
	Burghard & Paffrath (1927)	-	2 - 3 6 - 8	-	Healthy infants. " adults.
	Popper & Wosazek (1931a,b)	177 24*	0.2 - 8.5 1.6 - 6.2		24* of the 177 died suddenly without chronic disease.
	Haex (1944)	2	0.5 , 0.6	0.6	Jaundice.
	Illingworth & Cori (1952)	2	0.6 , 4.6	-	Infants; first had enlarged liver.
	Polglase et al (1952)	1 1		2.6 0.1	Heart disease Respiratory paralysis.

CHAPTER. III

ANALYTICAL METHODS.

A. DETERMINATION OF THE METABOLIC MIXTURE IN HUMAN SUBJECTS.

To calculate the metabolic mixture for a given period it is necessary to collect the following data:-

(1) the O_2 utilisation and CO_2 output;

(2) the urinary N;

(3) the time spent in representative activities throughout the period.

In the experiments described in Chapter IV of this thesis, the main activity was sitting, with short periods of walking at a measured rate and approximately 8h. in bed at night for the longer experiments. Incidental activities not classed as sitting, walking or in bed are included in the term "up and about".

Expired air samples were collected frequently throughout each experiment using a Douglas Bag for the resting samples and a Max-Planck respirometer for the exercise measurements. Each resting sample was collected for 7 to 10 min. after the subject had rested for at least 30 min., and the walking samples for 5 min. in the middle of a 20 to 30 min. controlled walk. Samples of expired air were analysed in duplicate using the Lloyd modification of the Haldane apparatus (Lloyd, 1958), with chromous chloride solution as oxygen absorbant (Dahlstrom & Wahlund, 1949). The oxygen utilisation (in ml./min.) and R.Q. were cal-

:culated for each sample and the mean values of these, for each activity during the period were determined. No measurements of respiratory exchange were made during the up-and about activities for which the oxygen con-:sumption was assumed to be twice the amount used while sitting and the R.Q. intermediate between sitting and walking.

The total time spent sitting, walking, in bed and up and about were recorded for each subject and these figures were used to calculate the total O_2 consumption and CO_2 output for the period. A typical example is given in Table 5.

Table 5 Oxygen utilization and carbon dioxide output of subject Nas. for 24 h.

Activity	Time (min)	O_2 used (ml./min.)	O_2 used (total litres)	CO_2 output (ml./min.)	CO_2 output (total litres)
Lying	406	211	85.7	192	77.8
Sitting	922	247	227.7	225	207.4
Walking	52	1044	54.3	950	49.4
Up & about	60	500	30.0	455	27.3
	1440		397.7		361.9

Urinary N 10.6 g./24 h.

The total urine was collected for each 24 h. (or shorter period), was tested qualitatively for glucose and its volume and specific gravity were recorded. Each

sample was diluted to a volume equivalent to an excre-:tion rate of 3 ml./min. and nitrogen was determined on 1 ml. aliquots by the Kjeldahl method using the catalyst recommended by Jacobs (1959).

The proportions of O_2 utilised and CO_2 produced will depend on the detailed composition of the particular metabolic mixture since the protein, fat and carbohydrate being metabolised will vary in their content of amino acids, fatty acids and monosaccharides respectively. It is therefore necessary to adopt mean values for the O_2 uptake and CO_2 and energy production per g. protein, fat and carbohydrate metabolised. The following figures used by Zuntz (1897) and Magnus-Levy (1907) have been adopted for calculating the metabolic mixture throughout this thesis:-

Substance Metabolised	O_2 used ml./g.	CO_2 produced ml./g.
Protein	966.3	773.9
Fat	2019.2	1427.3
Carbohydrate	828.8	828.8

Zuntz (1897) derived these figures by using empirical formulae for carbohydrate, animal fat and protein based on chemical analyses. The figures used by Zuntz for protein were obtained by analyses of meat and the determination of the losses of N and C in the urine and faeces of dogs consuming the meat. Using the above figures and assuming

that 1g. urinary N represents the metabolism of 6.25g. protein, the metabolic mixture was calculated:-

Protein = 6.25 x urinary nitrogen (Nu)

Carbohydrate = 4.12 CO_2 - (2.91 O_2 + 2.56 Nu)

Fat = 1.69 (O_2 - CO_2) - 1.94 Nu.

The metabolic water was calculated by multiplying the metabolic protein, carbohydrate and fat by 0.41, 0.60 and 1.07 respectively.

B. DETERMINATION OF CHANGES IN BODY WEIGHT AND THE WATER BALANCE IN HUMAN SUBJECTS.

In order to determine changes in body composition it is necessary to measure body weight by an accurate standardised procedure, and to calculate a water balance.

In the experiments lasting 24 h. the subjects were weighed naked at the beginning and end of the period. In the longer experiment the two women were weighed in light surgical gowns at 8.0 a.m. each day before breakfast. In both cases a metabolic beam balance accurate to ± 1g. was used and the weighing was immediately preceded by the voiding of urine.

The daily change in body weight = the weight of food and liquid consumed - the weight of urine and faeces - the insensible weight loss. The latter weight is made up of the evaporative water loss (in sweat and expired air) plus the difference in weight of the CO_2 output and the O_2 intake. (Density of O_2 = 1.4290g./l: density of CO_2 = 1.9769g./l.)

The daily change in body water = the water content of the food and liquid consumed,

+ the metabolic water

- the water content of the urine and faeces

- the evaporative water loss.

The water content of the food and faeces was determined by analysis (see Analytical Methods, section C below) and the water content of the urine was calculated

using the Trapp formula (Trapp, 1850; as quoted by Passmore, Strong & Ritchie, 1959):-

Urinary water = urinary weight - urinary solids.

Urinary solids (g./l)

= (1000 x specific gravity - 1000) x 2.

The water balance can also be expressed in the following manner:-

Δ Body water

= Δ body weight

+metabolic water

+urinary and faecal solids

+weight of (CO_2 - O_2)

-dry weight of the food intake.

C. ANALYSIS OF FOOD, FAECES, URINE, AND BLOOD FROM THE EXPERIMENTS ON HUMANS.

1. Food.

In the short experiments meals were served consisting of weighed portions of white bread, apricot jam, and tinned peaches. Samples of bread, jam and fruit were analysed separately.

In the longer experiment on 2 subjects, 3 portions of all meals were weighed out each day; 2 portions were fed to the subjects and the third portion was reserved for subsequent analysis. This third portion was homogenised in a Waring blendor and aliquots were taken for the estimation of the water content.

The foods and diets were dried in a vacuum oven at 80° C (70° for the jam and fruit), and the water content was calculated. The dried samples of the diets were then pooled for the appropriate periods and stored in a freezer. The following analyses were carried out on the dried foods and diets:-

(1) Fat was determined by Soxhlet extraction using anhydrous ethyl ether.

(2) Nitrogen was estimated by the Kjeldhal method and protein calculated as N x 6.25 (except for bread where the factor 5.7 was used).

(3) Carbohydrate was determined by the anthrone method of Trevelyan and Harrison (1952); the dried foods were digested with takadiastase and the colour pro-

:duced by the addition of anthrone reagent was read in a spectrophotometer at 620 mμ. Glucose and purified starch were used as standards and the re-:sults were expressed as polysaccharide.

(4) The gross energy value was measured in a bomb calorimeter.

(5) Total ash; the dried foods were heated in platinum crucibles in a furnace at 500° C.

2. Faeces.

In the hospital study the faeces for each subject were weighed daily and stored in a refrigerator. Each 3 to 4 days the faeces were pooled and homogenised. The water, fat, energy value and total ash were determined on each pooled sample as described above for the food anal-:ysis.

In the experiments lasting 24 h., faeces were passed by 4 subjects only. The faeces were weighed but not an-:alysed; in each case the stools were well formed and the water content was taken as 75%.

3. Urine.

The collection of urine and the determination of urinary nitrogen were described above (Analytical Methods section A.). The diluted urines were stored in a freezer.

Total urinary ketones were measured for the 2 over-:weight women by the method of Thin & Robertson (1952) as modified by Johnson, Sargent & Passmore (1958). The method is based on the oxidation of aceto acetate and

β - Hydroxybutyrate to acetone using potassium dichromate (in $H_2 SO_4$), with metaphosphoric acid acting as a de-:proteinising agent and a catalyst in the oxidation. The acetone formed was allowed to diffuse into alkaline sali-:caldehyde in Conway plates and the colour measured at 490 mμ in a spectrophotometer. Interfering compounds (including citrates and glucose) were removed by the method of Peters & Van Slyke (1932). A solution of a calcium zinc salt of β - hydroxybutyric acid, of a con-:centration of 1mM./l. was used as a standard.

4. Blood.

At the beginning and end of the experiments of Series 2, blood was taken by venipuncture, in heparinised syringes. It was centrifuged immediately and the follow-:ing estimations were made on the blood plasma.

(1) Glucose. The plasma (0.1 ml.) was diluted (0.4 ml. water) and 6% perchloric acid (0.5 ml.) was used as a deproteinising agent. The mixture was centrifuged and filtered and the filtrates stored in the freezer for subsequent glucose estimation. Preliminary checking re-:vealed no loss of glucose when the filtrate was stored in this way for 10 days. The glucose oxidase method of Huggett and Nixon (1957) was used, and the colour pro-:duced after incubation at 37° C (of 5 ml. enzyme reagent with 0.2 ml. stored filtrate) was read at 420 mμ in a spectrophotometer. Glucose solutions treated in the same manner were used as the standard.

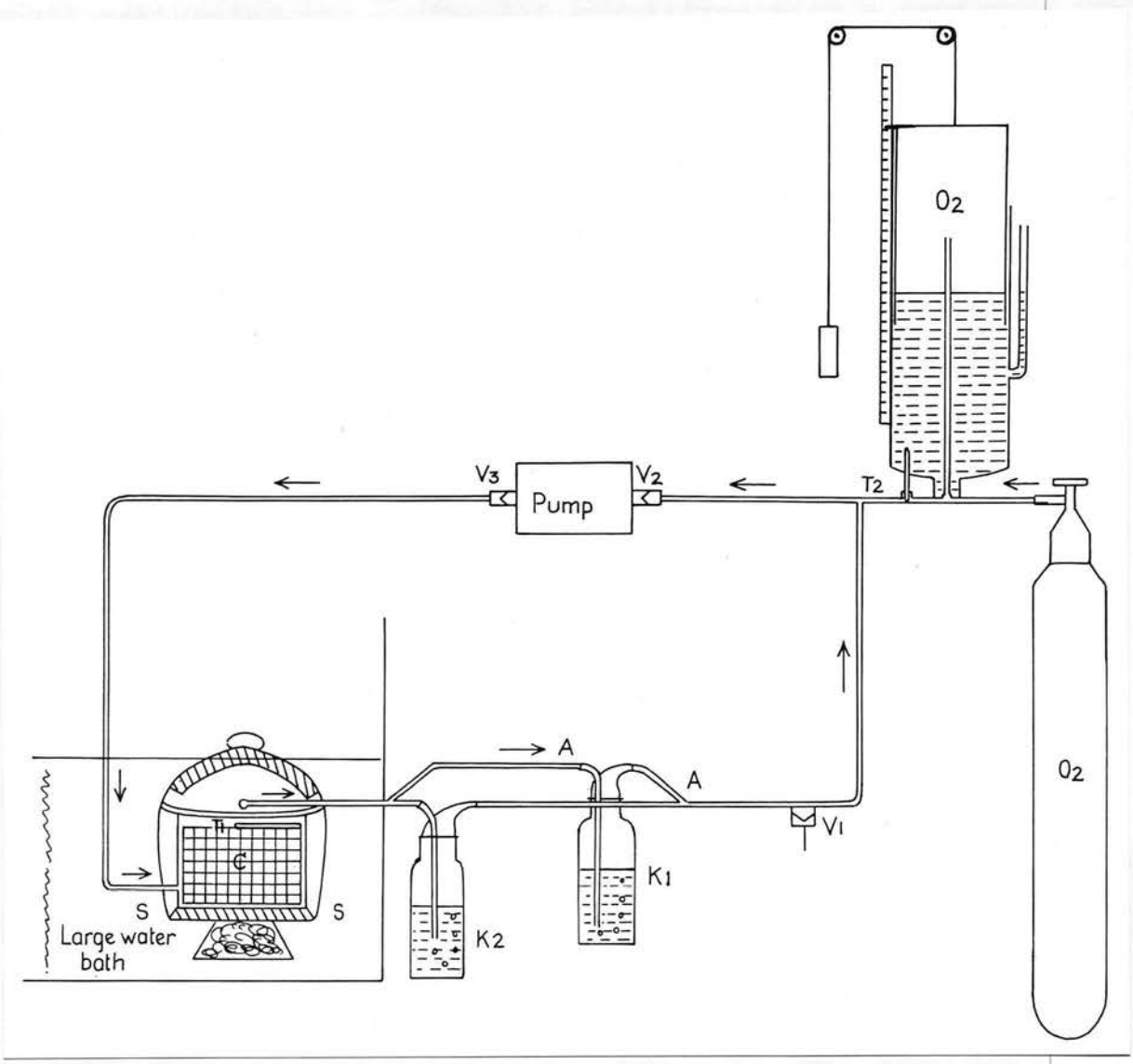
In the experiments of Series 3, finger-prick blood samples were taken and deproteinised and the glucose estimated as described above.

(2) Total ketones. The same method was used as for urinary ketones.

(3) Non-esterified fatty acids. (N.E.F.A.) Immediately following the centrifuging of the blood, N.E.F.A. was ex-:tracted from 0.5 ml. aliquots of plasma by the method of Dole (1956). A solution of recrystallised palmitic acid in heptane (1μM/ml.) was used as a standard, and 30 ml. isopropanol : 10 ml. heptane : 1 ml. N. H_2SO_4 as the ex-:traction mixture. Freshly prepared 0.02 N. NaOH solu-:tion was used for titration. Since heptane was the solvent for the standard solution, a volume of heptane equal to the volume of standard solution in use was added to the tubes containing plasma and blanks, in order to produce a heptane layer of the same volume in all tubes.

Fig. 1.

Diagram of the metabolic apparatus used for determining O_2 utilization and CO_2 output of rats.



D. METHODS USED IN EXPERIMENTS WITH RATS.

1. Oxygen utilization and carbon dioxide output.

A plan of the metabolism apparatus is given in Fig.1. The animal was placed in a wire gauze cage inside a small desiccator (c). The lid was fitted with a padded metal strip held firmly with spring clips (S) and the desiccator placed in a large water bath at room tempera-:ture. Oxygen was circulated from a cylinder and con-:trolled by valves (V1, V2, V3). Two bubbling flasks (K1, K2) with ground glass stoppers were placed in parallel and contained 0.5 N.KOH.

The apparatus was flushed with O_2 several times with V1 open and CO_2 being absorbed in K1. The spirometer was filled with O_2, V1 was closed and the circuit was switched to K2 by means of clips at A. Zero time was taken at the moment when A was closed. The temperature in the animal chamber and at the spirometer were recorded (T1, T2) and the level of the spirometer read at the end of 30 sec. A preliminary period of 45 min. was allowed by which time the animal chamber had reached a steady temperature.

At the end of the preliminary period K2 was replaced and 2 test periods of 45 min. each followed. The level of the spirometer was recorded for 44 min. and the tempera-:ture of the circulating oxygen was recorded from T2.

The perspex spirometer bell had a volume of 2200 ml and a height of 25 cm. A fall of 1 cm. therefore re-:presented 88 ml. O_2. The volume was corrected in each

case to that of 0°C and 760 mm. pressure saturated with water vapour.

To determine the CO_2 output, 90 ml. 0.5 N.KOH were used for each test and 10 ml. aliquots were taken for titration. The CO_2 was immediately precipitated by the addition of 1g. barium chloride to each aliquot and the excess KOH titrated with 0.5 N.HCl using phenolphthalein as indicator.

2. Tissue Glycogen.

The following procedure which is a modification of the method of Good, Kramer & Somogyi (1933) was used for determining the glycogen content in samples of liver, muscle and skin of rats.

0.5 - 1 g. tissue was placed in a weighed ice-cooled centrifuge tube containing 1.5 ml. 30% KOH and reweighed. The tubes were heated for 1h. in a boiling water bath, with occasional shaking. When cool, 1.7 ml. 95% ethanol was added and the solutions were mixed by stirring with a coarse platinum wire. The tubes were heated cautious-:ly until the alcoholic solution boiled, to coagulate the precipitated glycogen.

After standing overnight in the refrigerator, the tubes were centrifuged at 3000 r.p.m. for 20 min. The supernatant was poured off, the precipitate was washed with 2 ml. 60% ethanol and the centrifuging repeated. The supernatant was poured off and the tubes were heated for 5 min. in a boiling water bath.

To each tube 5 ml. o.55 N.HCl was added and the tubes (calibrated to 5 ml.) were heated in the boiling water bath for 2½ h. and the volume was rechecked. The solu-:tions were filtered and the following volumes of filtrate were added to 9 ml. water in glass-stoppered tubes to give dilutions suitable for glucose estimation:- muscle 1 ml.; liver 0.2 ml.; skin - no dilution was required. The glucose was estimated by the glucose oxidase method of Huggett & Nixon (1957) using as standards, solutions of 2, 5, 10 and 15 mg. glucose 100 ml.

CHAPTER. IV.

THE EXPERIMENTS.

A. EXPERIMENTS OF SHORT DURATION ON HUMAN SUBJECTS.

Three series of experiments were carried out using similar laboratory methods. The subjects were all healthy students, technicians or research workers and none were obese or exceptionally thin. In between meals the subjects sat and read in easy chairs, except for brief periods when the respiratory exchanges were being measured while walking on a treadmill. In the experi-:ments lasting through the night, the subjects slept on camp beds in the laboratory.

The meals consisted of white bread, jam and a little tinned fruit, chosen to provide sufficient protein, very small amounts of fat but large quantities of carbohydrate. Tea was provided to drink, with sugar but no milk; water intake was unrestricted but accurately measured. The subjects were encouraged to eat as much as possible at each meal and the calorie intake was always in excess of the measured energy expenditure.

Full details of the results are given in the Appen-:dix (Tables 24 to 33) but the mean results of each ex-:periment are summarised in the present chapter.

Series 1. The subjects, 3 men and 5 women, received 4 meals at 3 hourly intervals and their respiratory ex-:changes were followed for 14 h. Table 6 shows the ranges and means of the R.Q.'s after each meal, and Table 7 the

mean values for the total intake each day and the cal-:culated metabolic mixture. It will be seen that the mean values for the resting R.Q.'s did not rise above 0.94 and that despite the very low dietary intake of fat and the excess dietary calories, fat still appeared to be mobilised and used. For one subject the experiment was continued for 32 h. and his maximum R.Q. was 0.96, 28 h. after the commencement of the experiment.

Series 2. In the second series the dietary intake was increased and the period of observation was 24 h. The subjects, 9 men, 2 of whom had been in the first series, were given 5 meals and were encouraged to eat to their maximum capacity. This varied greatly from 2610 to 5120 kcal./day. Table 8 gives the ranges and means of the R.Q.'s after each meal and throughout the night and early morning; a maximum mean R.Q. of 0.99 was recorded at 2.0 a.m. Table 9 shows the mean values for the dietary intake and the metabolic mixture. In Table 10 the change in body weight of each subject has been expressed in g./65 Kg. Only in subject Wil. who ate 1.17 kg. of carbohy-:drate was there demonstrated a net conversion of carbohy-:drate to fat. The mean values(in mM./l. $\pm$ standard error (S.E.)) for blood glucose, total ketones and non-:esterified fatty acids (N.E.F.A.) at the start and end of the experiment were (see Table 33) :-

	Glucose	Ketones	N.E.F.A.
Start	4.61 (±0.20)	0.20 (±0.02)	0.75 (±.07)
End	4.74 (±0.13)	0.12 (±0.01)	0.54 (±.06)

Series 3. In order to test whether a longer period of time was required to obtain R.Q.'s above 1.0 following an excess intake of carbohydrate, a series of experiments lasting 8 days was planned. In these, Dr. Passmore and the writer acted as subjects. The response to a large mid-day meal of carbohydrate was studied for a period of 6 h. during 8 days. For the first 2 days we ate our normal diets, but for the next 4 days we restricted the fat intake but ate according to normal appetite. For the final 2 days we endeavoured to exclude fat from the diet and we ate carbohydrate to our maximum capacities. Table 11 shows the mean of the R.Q.'s after the test meal. It will be seen that even on day 8 this did not rise above 1.0. The calculated metabolic mixture (Table 12) in-:dicates that even after this longer period of restriction of fat and forcing of carbohydrate, conversion of carbohy-:drate to fat was insignificant. During the 8 days R.P. gained 0.3 kg. in weight and Y.S. gained 2.8 kg. The glucose content of blood (from a finger prick) was measured at the start and end of each 6 h. period. (Table 33.) With R.P. an increased level of blood glucose at the end of the period resulted only on the last 2 days of the experiment. With Y.S a rise was observed on days 2,

5 and 6 but not on days 7 and 8. The very high level for Y.S at the start on day 8 was due to continuous carbohy-:drate feeding on the morning of that day.

Table 6.

The Respiratory Quotients at rest and when walking after 4 successive meals rich in carbohydrate and low in fat.

	At rest		Walking	
	Range	Mean	Range	Mean
Basal	0.77 - 0.85	0.82	-	-
Before meal 1	-	-	0.83 - 0.87	0.85
After meal 1				
1 hr.	0.86 - 0.96	0.90	-	-
2 hr.	0.81 - 0.92	0.88	0.91 - 0.96	0.93
After meal 2				
1 hr.	0.88 - 1.00	0.92	-	-
2 hr.	0.85 - 1.01	0.93	0.90 - 0.97	0.95
After meal 3				
1 hr.	0.85 - 0.96	0.92	-	-
2 hr.	0.90 - 0.97	0.93	0.92 - 0.96	0.95
After meal 4				
1 hr.	0.84 - 1.02	0.94	-	-
2 hr.	0.89 - 0.97	0.94	0.92 - 0.99	0.96

Table 7.

The diet, the metabolic mixture and the proportions of the mixture derived from protein, carbohydrate and fat during 14 hrs. with meals rich in carbohydrate and low in fat.

	Protein	Carbohydrate	Fat	Calories
Diet g.	45	421	2	1870
Metabolic mixture g.	36	242	31	1430
Percentage of calories derived from	10	70	20	

(mean values for 8 subjects)

Table 8.

The Respiratory Quotients at rest and when walking during a 24 h. period, when receiving excess of a diet rich in carbohydrate.

Day	Approximate times	At rest range	At rest mean	Walking range	Walking mean
1	9.00	0.73 - 0.86	0.80		
	Meal				
	11.00	0.78 - 0.94	0.88	0.89 - 1.01	0.95
	Meal				
	14.00	0.75 - 1.01	0.90	0.90 - 0.99	0.95
	Meal				
	17.00	0.84 - 1.05	0.92	0.90 - 1.00	0.95
	Meal				
	20.00	0.88 - 1.06	0.96	0.92 - 1.01	0.96
	Meal				
	23.00	0.85 - 1.03	0.96	-	-
2	2.00	0.87 - 1.09	0.99	-	-
	5.00	0.87 - 0.98	0.95	-	-
	8.00	0.77 - 0.98	0.88	-	-

Table 9.

The diet, the metabolic mixture and the proportions of the mixture derived from protein, carbohydrate and fat during 24 h. when receiving excess of a diet rich in carbohydrate.

	Protein	Carbohydrate	Fat	Calories
Diet g.	86	884	3	3910
Metabolic mixture g.	76	452	30	2440
Percentage of calories derived from	13	75	12	

(mean values for 9 subjects)

Table 10.

Series 2. The changes in body weight and composition (in g./65 kg) of the 9 men who ate 5 meals containing large quantities of carbohydrate in excess of their calorie needs. The results are arranged in decreasing order of calorie excess (k cal).

	Body Weight	Water	Protein	Fat	Carbo-hydrate	Calorie
	Δ	Δ	Δ	Δ	Δ	excess
Har.	538	-40	-3	-21	600	2270
Nim.	1636	1130	25	3	480	2110
Wil.	1225	740	36	15	440	2080
Pas.	1518	1060	-9	-38	510	1680
Ste.	415	70	4	0	340	1420
Nas.	1439	1060	16	-41	400	1340
Pic.	446	160	-6	-18	310	1070
New.	11	-240	11	-69	310	670
Bow.	-350	-560	-10	-105	330	320

Table 11.

The Respiratory Quotients after a heavy meal rich in carbohydrates and low in fat. Mean figures for 8 observations each day taken over a 6 h. period.

Day	Previous diet	Subjects Y.S.	R.P.
1	Normal	0.89	-
2	Normal	0.90	-
5	Reduced fat	0.92	0.94
6	Reduced fat	0.92	0.94
7	Excess carbohydrate	0.94	0.95
8	Excess carbohydrate	1.00	0.96

Table 12.

The metabolic mixture over a 6 h. period after a meal rich in carbohydrate. For the previous 2 days the subjects had eaten carbohydrates in excess of caloric needs.

	Protein	Carbohydrate	Fat
Meal g.	24	204	1
Metabolic mixture g./6 h.			
Y.S.	16	111	-5
R.P.	15	102	5

B. AN EXPERIMENT OF LONGER DURATION ON HUMAN SUBJECTS.

Two women who had been attending an obesity clinic but were otherwise healthy agreed to take part in this study. The age, height and weight of each subject is given in Table 13 below.

Table 13

The age, height, weight, basal metabolic rate (B.M.R.) and total body water (measured by the tritium oxide dilu-:tion method) are given for the two women.

			BODY WEIGHT		B.M.R.		
	AGE years.	HEIGHT cm.	initial kg.	as % of standard (Odier & Mach, 1949)	measured ml.O_2/min.	as % of standard (Robertson & Reid, 1952)	TOTAL BODY WATER l.
Betty	35	167	91.9	146	271	116	42.0
Pat	25	168	92.8	146	271	116	44.5

Both had previously been much fatter; Betty weighed 118 kg. and Pat 112 kg. when they first attended the clinic 15 months previously. They each lost 21 kg. dur-:ing the first 9 months of weight reduction, after which further decreases had become more difficult.

The experiment lasted 23 days during which the sub-:jects slept in a hospital ward, but spent the day in a

room set aside in the laboratory. Their time was spent reading, watching television and doing light handicrafts. They went out for formal exercise consisting of about one hour's walking each day; half of this exercise was taken on a constant route along a flat field, during which samples of expired air were collected. The 2 women kept a diary record of their activities and this was frequently checked. Measurements of energy expenditure were made on 17 of the 23 days except for the B.M.R. which was measured each day. As is usual with obese people the B.M.R. was high (+16% during the control period) when compared with standards based on surface area (Robertson & Reid, 1952). The total body water of 42.0 and 44.5 l. is equivalent to a lean body mass of approximately 60 and 63 kg., so muscle and adipose tissue both contributed to the excess weight.

For the first 9 days they were on a control diet which approximately met their calorie needs, for the next 9 days they were overfed but for the last 5 days they re-:ceived only a token diet. The diets were composed of common foods, chosen after discussion with the patients who agreed to eat the total food presented at each meal. During the overfeeding period, additional bread, jam and sweetened fruit juices were provided and the subjects were persuaded to take as much as possible of these. Records of the amounts consumed were kept and each of these extra foods was analysed separately. No food was eaten on the first day of the underfeeding period, and only one meal on

the second day with 2 small meals on each of the remaining days.

The analytical data collected during this experiment have been tabulated. The detailed results are given in the Appendix:-

Table 34 Daily weight balance

35 Diets

36 Faeces

37 Urine

38 Oxygen Consumption

39 Respiratory Quotients

40 Daily Activities

41 Metabolic Mixtures

In the present chapter mean values, calculated from the tables above, are included.

Fig 2. indicates the daily changes in body weight while Table 14. gives the changes for each period, and the weights of the fluid and food intakes and of the outputs of urine and faeces. Betty normally drank a large amount of water; throughout the whole experiment the water intake was unrestricted but carefully measured. The mean in-:sensible weight losses were the same in the control and overfeeding periods (1522 $\pm$ 51 and 1568 $\pm$ 47 for Betty; 1550 $\pm$ 62 and 1674 $\pm$ 79 for Pat) but showed a significant fall for both subjects during underfeeding (1155 $\pm$ 106 and 1177 $\pm$ 77 for Betty and Pat respectively).

Tables 15 and 16 give the mean values for the oxygen

utilization and R.Q.'s. The mean values designated "in bed" include the basal values, in order to give figures which are more representative for the whole post-absorptive period during the night. In addition the basal values have been included separately. The basal O_2 consumption remained constant throughout the experiment except for Pat in the underfeeding period when the volume ($\pm$S.E.) rose to 299$\pm$ 7 ml./min. (compared with 271$\pm$5 and 278$\pm$4 ml./min. in the control and overfeeding periods respectively). The corresponding volumes for Betty for these 3 periods were 272$\pm$5, 271$\pm$5 and 270$\pm$6 ml./min.

The daily metabolic balances for each period are summarised in Tables 17 - 19. The tables show the intake of protein, fat, carbohydrate and water and the output of these in faeces and urine and through the skin and lungs. The calculated metabolic mixtures and energy balances are also included. The daily cutaneous loss of 1g. protein has been assumed (Darke, 1960). During the control period the small loss in weight was approximately equal to the negative water balance in each case. For the overfeeding and underfeeding periods the change in body composition is presented graphically in Figs. 3 and 4.

Total ketones were determined in pooled samples of urine for the control and overfeeding periods for each subject, but in daily samples during the underfeeding period. The results are given in Fig. 5. In spite of the steep rise during calorie restriction, only the final

value for Betty (5.85 μM/min.) is greater than the upper level found in normal conditions in the healthy young men studied by Johnson, Sargent & Passmore (1958).

The changes in body composition calculated from the daily metabolic balances are presented in Table 20. During overfeeding Betty gained more weight than Pat, for a smaller excess of calories. This can be attributed to a greater retention of water. Pat retained more protein and appeared to store more carbohydrate. This latter result reflects a slower rise in R.Q. for Pat and a larger carbohydrate intake.

Fig. 2.

The body weight (in kg.) of 2 overweight women during 9 days on a control diet, 9 days of overfeeding and 5 days of underfeeding.

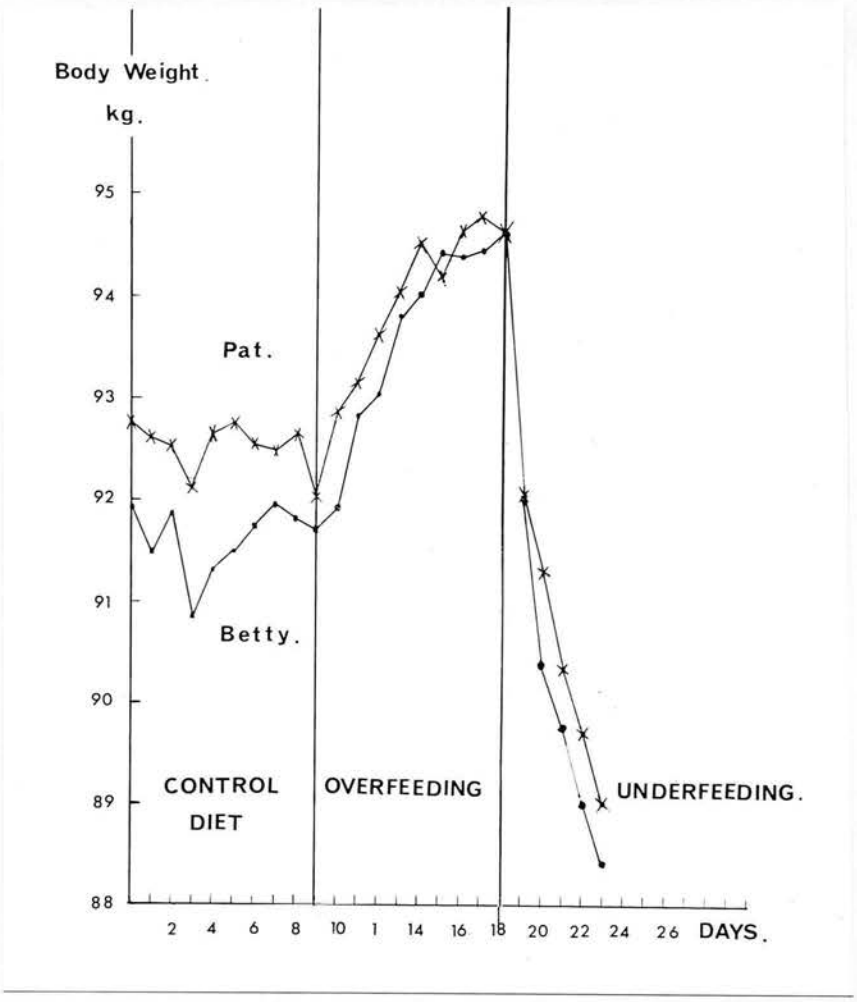


Table 14.

Mean values for the change in body weight, the intake of fluid and food, the output of urine and faeces and the insensible weight loss (all in g./day) for the control, overfeeding and underfeeding periods.

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
Δ Body weight	- 16	- 78	318	287	-1243	-1126
Intake						
Water drunk	2145	816	1987	710	2290	1307
Food eaten	2526	2526	3079	3143	471	471
Output						
Urine	3004	1784	2988	1788	2817	1664
Faeces	161	86	192	106	32	63
Insensible weight loss	1522	1550	1568	1674	1155	1177

Table 15.

Mean values for the oxygen consumption (in ml/min) determined by analysis of expired air collected during representative activities at irregular intervals throughout the day and night. The basal oxygen consumption was measured at the end of each 24h period.

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
In bed	268	281	293	295	281	307
Sitting	320	324	333	335	278	299
Walking	1150	1183	1190	1185	1037	1085
Basal oxygen consumption	271	271	270	278	272	299

Table 16.

Mean values for the RQ's for the control, overfeeding and underfeeding periods.

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
In bed	.84	.86	1.00	.96	.80	.77
Sitting	.87	.88	.95	.95	.86	.84
Walking	.83	.81	.91	.92	.79	.77
Basal R.Q.	.84	.86	.94	.93	.80	.76

Table 17.

The mean daily intake, output and utilization of energy, protein, fat, carbohydrate and water and the change in body weight during a control period of 9 days.

A. Betty

	Energy kcal	Protein g	Fat g	Carbo-hydrate g	Water g	Body weight g
Diet	3390	131	147	297	4056	
Faeces	210	12	10		120	
Urine	150				2949	
Skin & lungs		(1)			1367	
Metabolised	2970	117	124	315	370	
BALANCE	+60	+1	+13	-18	-10	-16

B. Pat

	Energy kcal	Protein g	Fat g	Carbo-hydrate g	Water g	Body weight g
Diet	3390	131	147	297	2727	
Faeces	100	7	3		63	
Urine	150				1723	
Skin & lungs		(1)			1388	
Metabolised	3080	118	128	330	383	
BALANCE	+60	+5	+16	-33	-64	-78

Table 18.

The mean daily intake, output and utilization of energy, protein, fat, carbohydrate and water and the change in body weight during overfeeding for 9 days.

A. Betty

	Energy kcal	Protein g	Fat g	Carbo-hydrate g	Water g	Body weight g
Diet	4380	145	91	645	4139	
Faeces	270	18	11		141	
Urine	140				2940	
Skin & lungs		(1)			1280	
Metabolised	3160	110	24	598	430	
BALANCE	+810	+16	+56	+47	+208	+318

B. Pat

	Energy kcal	Protein g	Fat g	Carbo-hydrate g	Water g	Body weight g
Diet	4600	146	91	698	2872	
Faeces	130	10	3		78	
Urine	140				1736	
Skin & lungs		(1)			1392	
Metabolised	3170	109	30	586	429	
BALANCE	+1160	+26	+58	+112	+95	+287

Table 19.

The mean daily intake, output and utilization of energy, protein, fat, carbohydrate and water and the change in body weight during underfeeding for 5 days.

A. Betty

	Energy kcal	Protein g	Fat g	Carbo-hydrate g	Water g	Body weight g
Diet	350	14	11	35	2694	
Faeces	50	4	2		22	
Urine	90				2794	
Skin & lungs		(1)			1056	
Metabolised	2630	68	153	217	322	
BALANCE	-2420	-59	-144	-182	-856	-1243

B. Pat

	Energy kcal	Protein g	Fat g	Carbo-hydrate g	Water g	Body weight g
Diet	350	14	11	35	1711	
Faeces	70	6	2		48	
Urine	100				1639	
Skin & lungs		(1)			1096	
Metabolised	2940	78	198	179	351	
BALANCE	-2770	-71	-189	-144	-721	-1126

Fig. 3.

The change in body composition of 2 overweight women receiving excess calories for 9 days.

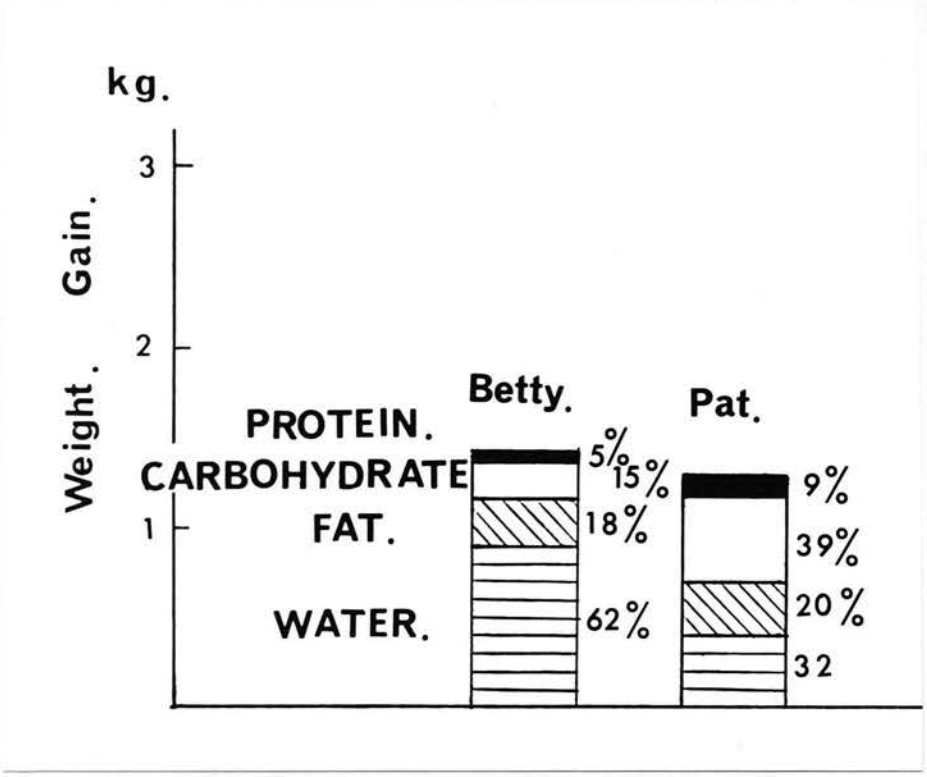


Fig. 4.

The change in body composition of 2 overweight women during 5 days of underfeeding.

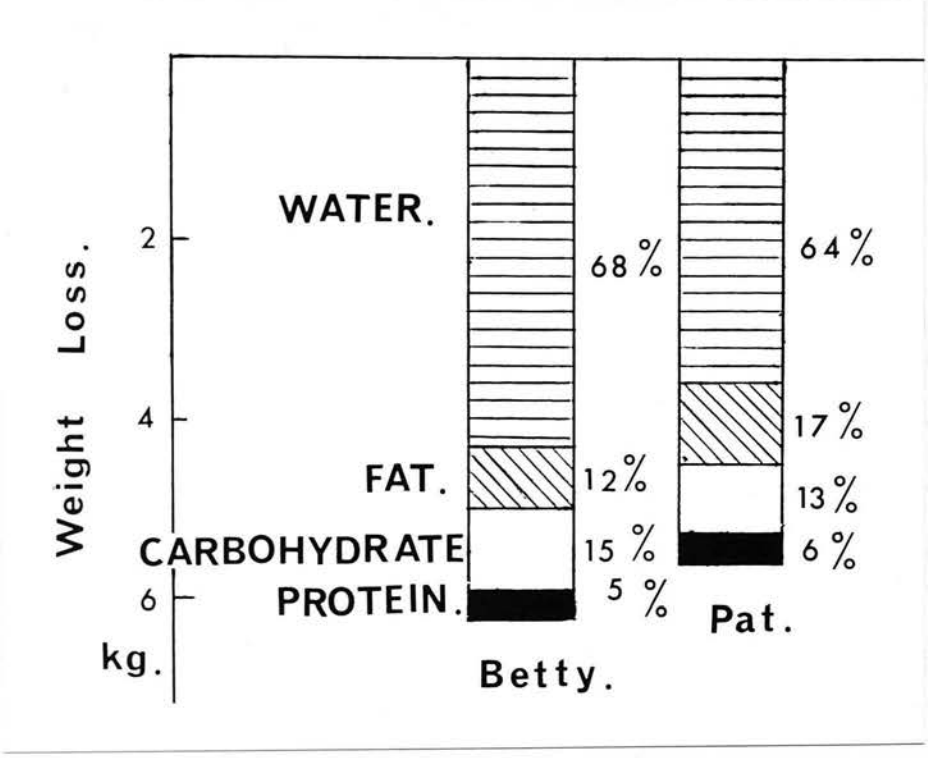


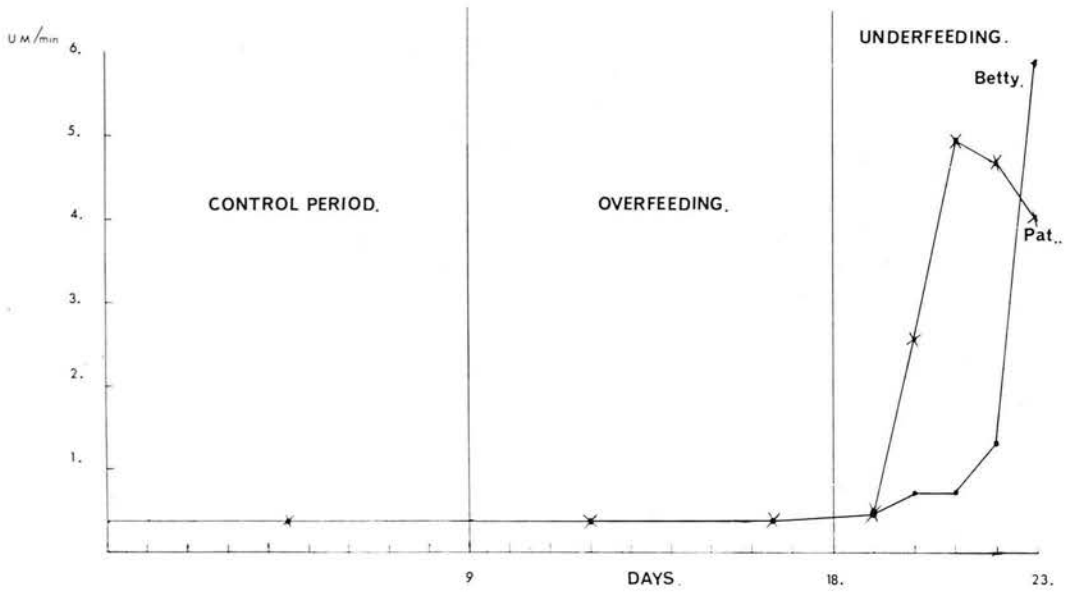
Table 20.

The total changes in body weight and body composition (in kg) and the calorie balance (in kcal) during 9 days on a control diet, 9 days of overfeeding and 5 days of underfeeding.

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
Body weight	-0.14	-0.70	+2.86	+2.58	-6.22	-5.63
Body composition						
Protein	+0.01	+0.05	+0.14	+0.23	-0.30	-0.36
Fat	+0.12	+0.14	+0.50	+0.52	-0.72	-0.95
Carbohydrate	-0.16	-0.30	+0.42	+1.01	-0.91	-0.72
Water	-0.09	-0.58	+1.87	+0.86	-4.28	-3.61
Calories	+540	+540	+7290	+10440	-12100	-13850

Fig. 5.

The urinary excretion of ketones of 2 overweight women during 9 days on a control diet, 9 days of overfeeding and 5 days of underfeeding.



C. EXPERIMENTS ON RATS.

In the experiments on human subjects already described, one possible explanation for the slow rise in R.Q. after an excess intake of carbohydrate would be the storage of carbohydrate as glycogen prior to its con-:version to fat. In order to test this hypothesis an experiment using rats was planned in which the glycogen stores were measured following different dietary treat-:ments the aim of which was to produce a range of R.Q.'s The measurement of oxygen consumption and R.Q.'s in duplicate required over 2 h. (ad described in Chapter III section D). Immediately after this measurement the animals were stunned and the tissues were rapidly dis-:sected. Duplicate samples of abdominal and femoral muscles of liver and skin (from the abdominal area) were taken for analysis.

White male rats weighing 200 - 300 g., fed a stock rat cake, with no starvation period prior to the experi-:ment, were divided into 4 groups. The animals of the first 2 groups served as controls for the feeding experi-:ments. The animals were brought to the laboratory at 9.0 a.m. on the experimental day and given the treatment indicated below after which their R.Q. was measured before they were killed for the estimation of tissue glycogen. The times at which the R.Q. measurements were commenced were:-

Group 1 : 3.0 p.m., after 6 h. fasting;

Group 2 : 9.0 a.m., with no fasting or extra feeding;

Group 3 : 12.15 p.m., after being tube-fed twice (at 9.0 a.m. and 12 noon)

Group 4 : 9.15 a.m., immediately following tube-feeding. The animals in group 4 had also been tube-fed on the 2 previous days and extra glucose had been added to their diet.

70% glucose solution was used for tube-feeding at a level of 1g./100g. body weight for each feed.

In the Appendix Table 42, gives the O_2 utilization, CO_2 output and R.Q.'s of each rat and Table 43 the % glycogen content of the tissues. The mean values for the R.Q.'s and tissue glycogen for each group of rats are given in the present chapter (Table 21). In each group the glycogen content of abdominal muscle was greater than that of leg muscle. For liver, there was a range of values within each group as would be expected in an ex-:periment which was not preceded by a starvation period. The % glycogen in the muscle and liver of the rats in groups 3 and 4 was greater than the levels found in groups 1 and 2. In Fig. 6 the mean values for muscle glycogen have been plotted against the mean R.Q.'s for each group.

Table 21.

Mean values for the R.Q.'s, and the glycogen content of muscle, liver and skin (in g./100g each tissue) in 4 groups of rats with different carbohydrate intakes. (means ± S.E. are given)

Group	R.Q.	Glycogen			
		Leg muscle	Abdom. muscle	Liver	Skin
1	.81 (±.01)	.24 (±.01	.34 (±.4)	2.1 (±.4)	.05 (±.01
2	.84 (±.02)	.26 (±.01	.31 (±.02)	1.6 (±.4)	.03 (±.01
3	.95 (±.02)	.42 (±.01)	.52 (±.01)	4.1 (±.4)	.14 (±.02)
4	1.03 (±.01)	.54 (±.06)	.73 (±.09)	5.9 (±.6)	.13 (±.04)

Fig. 6.

The mean % glycogen of muscle tissue of 4 groups of rats receiving different amounts of dietary carbohydrate.

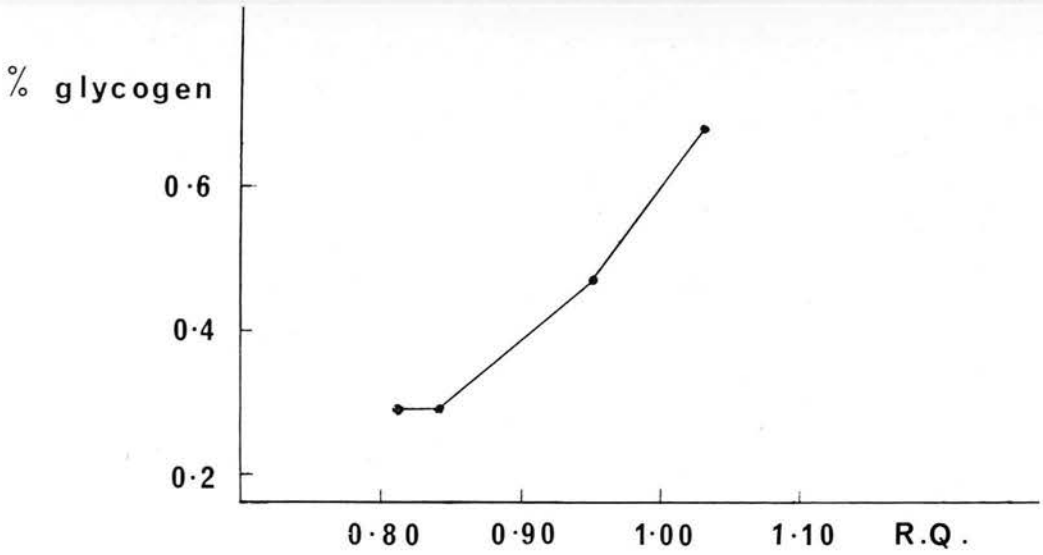
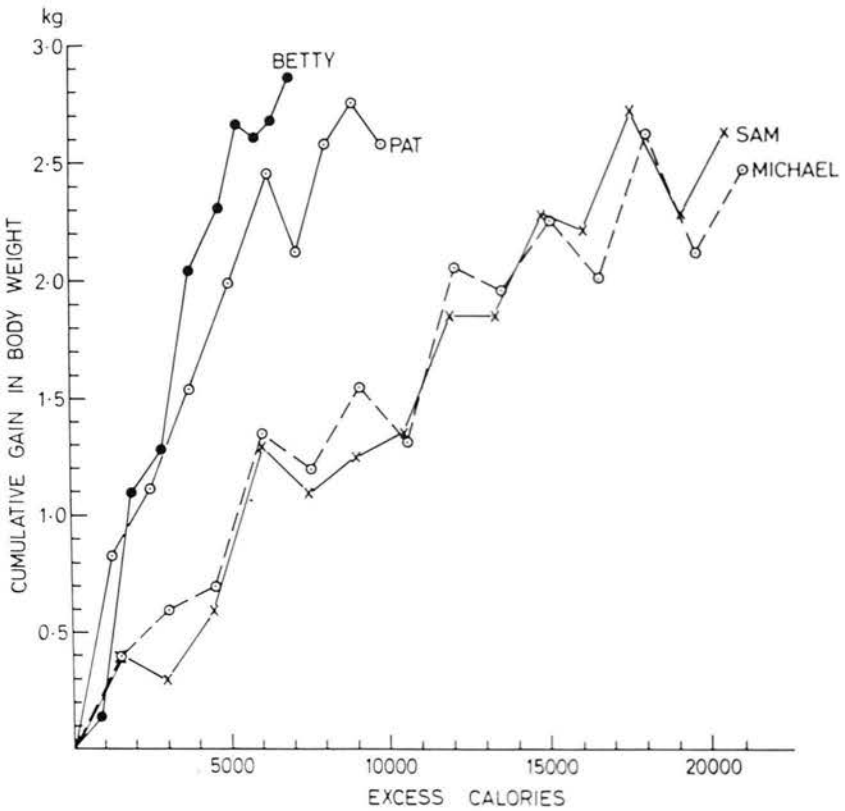


Fig. 7.

The cumulative gains in body weight of 2 fat women and 2 thin men related to the excess caloric intake during overfeeding.



DISCUSSION

In the present investigation experiments have been reported in which various effects of an excess caloric intake have been studied with human subjects. By calculating the composition of the metabolic mixture and comparing it with the net dietary intake an estimate has been made of the changes in body weight and composition in terms of protein, fat, carbohydrate and water. In the longer experiment with 2 overweight women, the period of caloric excess was followed by an energy defecit for 5 days.

Both in the experiments reviewed in Chapter II and those reported here there are large individual differences in the rate of weight gain. Often these differences cannot be adequately compared either because the energy expenditure is unknown or because the caloric intake or water balance have not been determined. Even when the energy and water balances are determined as accurately as possible differences still occur in the rate of gain in body weight when this is related to the caloric excess. The present investigation has used methods similar to those of Passmore et al. (1955 a,b) when thin subjects were studied. In Fig. 7 are shown the cumulative gains in body weight of Betty and Pat and of 2 of the thin men, plotted against the cumulative excess calories. Each point represents a daily change in

body weight. Betty gained 2.86 kg. (2.6 kcal/g.) and Pat gained 2.58 kg. (4.0 kcal/g.). The corresponding figures for Michael and Sam were 2.45 kg. (9.3 kcal/g.) and 2.62 kg. (8.3 kcal/g.). The chief difference between the fat women and the thin men was in water retention which was very small for the thin men during overfeeding. A similar finding was reported by Brozek, Grande, Taylor, Anderson, Buskirk & Keys (1957) when 13 men were overfed for 19 days after a previous low-calorie diet. For the first 7 days of overfeeding it was calculated by Brozek et al. (1957) that the gain in weight was 40% water, but for days 17 to 19 there was no gain in weight which was interpreted as a negative water balance masking the gains of fat and protein, but they did not measure the water balance.

In the shorter experiments reported in this Thesis the individual differences in weight gain in Series 2 (Table 10) were not surprising. Large day-to-day variations in body weight are commonly observed (Durnin, 1961; Adam, Best & Edholm, 1961) and are also indicated for the 4 subjects represented in Fig. 7. The present results confirm previous findings that short-term changes in body weight are usually closely related to changes in body water. In Series 2 only 4 of the subjects retained substantial amounts of water while Bow. who lost weight during overfeeding showed a negative water balance of 560g./65 kg. (Table 10). The same relationship between

body weight and body water was observed in Series 3 where Y.S. gained 2.8 kg. in 8 days. In the 3 days following the experiment there was a weight loss of 3 kg. and the urine volume was 2 l. greater than the fluid intake.

During underfeeding for 5 days Betty lost 6.22 kg. (1.9 kcal/g.) and Pat lost 5.63 kg. (2.5 kcal/g.); on the first day with complete starvation the losses in weight were 2.65 and 2.34 kg. respectively. The low figures for the caloric value of the tissue lost are due to the large negative water balance (4.37 kg. for Betty and 3.73 kg. for Pat). The loss of body water which occurs with obese people on a reducing regime is a well known fact, (Passmore, 1961). When Luethje (1902) fed a token diet to a 60 kg. woman who had previously been overfed she lost 6.02 kg. in 9 days.

The evaporative water loss. This accounts for a large proportion of the daily water balance, and of the loss of heat since the latent heat of vaporisation of water is 0.58 kcal/g. Newburgh, Wiley & Lashmet (1931) observed that the loss of heat by this means showed a constant relationship to the daily energy expenditure. They observed that the % of the energy lost by vaporisation by 7 subjects who were studied for long periods was 23.8 to 25.2, provided that the environmental temperature was moderate and the energy expenditure limited. When the diet and experimental conditions were

more highly controlled the range was 24.2 to 24.8%, based on an indirect calculation of the energy expendi-:ture. Newburgh, Johnston, Lashmet & Sheldon (1937) and Johnston & Newburgh (1942) tested the hypothesis further by direct measurement of energy expenditure with subjects in a chamber for 24 h. For 9 subjects the results ranged from 21.2 to 27.7% but the mean for each subject fell within the limits of 24.0 to 25.8%.

These findings of Newburgh et al. confirmed earlier measurements of evaporative heat loss. Atwater and Benedict (1903) in 14 experiments on 4 subjects found values of 21.7 - 25.0% with a mean of 24.2. Soderstrom & Du Bois (1917) reported values of 21 - 28% with 28 measurements by direct calorimetry.

In the experiments with the 2 overweight women re-:ported in this thesis the % of the total energy lost by vaporisation was in the same range as found by Newburgh et al. (1937) and the mean values for the whole experi-:ment were 24.7 for Betty and 24.8% for Pat. For the 3 periods the following percentages were calculated:-

	Betty	Pat
Control	26.7	26.1
Overfeeding	23.5	25.4
Underfeeding	23.3	21.6

The nitrogen balance. This was influenced by the periods of overfeeding and starvation. N retention following overfeeding (as discussed in Chapter II) was also found here; in 9 days Betty and Pat retained 23

and 37 g. N respectively. It takes a considerable period of time for the body to adjust to an increased intake of protein and calories. In experiments of this kind, both the increased protein intake and the sparing effect of the extra carbohydrate intake would influence the rate of protein catabolism.

During underfeeding the nitrogen loss was greater than the previous nitrogen retention but the caloric defecit was also greater than the caloric excess. In 5 days Betty lost 48 g. N and Pat lost 56 g; for a comparable period the N loss, in the experiment of Luethje (1902) discussed above, was 39 g.

Gains and losses in fat and carbohydrate. In interpreting the results of overfeeding experiments in man it is frequently assumed that the effects on the carbohydrate stores are negligible (Wiley & Newburgh, 1931; Passmore et al., 1955b; Keys et al., 1955), and that the gain in body weight represents the algebraic sum of the changes in water, protein and fat. As indicated by Table 18 Betty and Pat gained 56 and 58 g. fat/day respectively during the overfeeding period but also 47 and 112 g. carbohydrate. This finding is likewise supported by all the shorter experiments because it is based on the interpretation of the R.Q's.

Figs. 3 & 4 indicate that the tissues gained and lost by Betty were of similar composition and this is supported by Keys & Brozek (1953) and by Behnke, Osserman

& Welham (1953) from measurements of body density and total body water. Entenman, Goldwater, Ayres & Behnke (1958) confirmed this finding by analysis of adipose tissue of 2 men before and after weight reduction. However, for Pat who had gained less water than Betty the tissues gained and lost were not of the same composition, though for the 2 women the tissues lost were similar (Fig. 4).

In spite of the large excess intake of carbohydrate in all the experiments, the utilization of fat was only slowly suppressed. The mean values for the percentage of energy derived from fat were calculated as:-

Series 1	=	14 h.	20%
Series 2	=	24 h.	12%
Pat and Betty	=	9 days	8%

During the control period Pat and Betty had derived 39% of their calories from fat. The extent of the suppression of fat mobilisation is also indicated by the analysis of blood plasma in Series 2, though these values depend both on rates of production and utilization of the metabolites. The total ketones of blood fell from 0.20(±0.02) to 0.12(±0.01) mM/l. and the N.E.F.A. from 0.75(±0.07) to 0.54(±0.06) mM/l. in 24 h. Subject Bow. mentioned above for his loss of weight during overfeeding, derived 40% of his calories from fat (Table 32) and his high level of blood ketones changed from 1.42 - 0.29 mM/l. in 24 h.

During underfeeding fat supplied 55 and 63% of the calories for Betty and Pat respectively and the ketone excretion rose more rapidly for Pat than for Betty (Fig. 5). The ketone excretion during this period was not sufficient to disturb the well-being of the subjects and was in line with the resistance to ketosis often found in obese people (Strong, Passmore & Ritchie, 1958; Kekwick, Pawan & Chalmers, 1959).

It was calculated that Pat had stored 1.01 kg. carbohydrate during overfeeding, representing a store of 4140 kcal. This would be sufficient to meet the energy defecit of 2770 kcal./day for only 1½ days of the underfeeding period. For both subjects nearly all the RQ. values were less than 0.80 after 2 days of under-:feeding.

The finding of this thesis is the fact that it appears possible to increase the stores of carbohydrate in the human body for short periods by greater amounts than Soskin & Levine (1952) calculated would normally be present. This conclusion is largely based on the evidence of indirect calorimetry and it is therefore necessary to consider in some detail the validity of the classical interpretation of R.Q. values for human sub-:jects.

The interpretation of R.Q's. in man. For many years the R.Q. has been used as a numerical expression for the composition of the metabolic mixture. A single R.Q.

reading may represent the sum of 3 processes:-

(1) the relative combustion rates of protein, fat and carbohydrate;

(2) the sum of the interconversions of these three proximate principles in different organs of the body;

(3) the result of any change in the bicarbonate content of the tissue fluids, secondary to variations in acidity.

During hyperventilation or acidosis, the uncorrected R.Q. cannot be used as a measure of metabolic change.

In 1927 Cathcart and Markowitz questioned the validity of R.Q. measurements pointing out that the R.Q. varied with different carbohydrates. After the oral administration of 50 g. glucose to a man the R.Q. rose slowly to less than 1.0 whereas with sucrose, fructose or galactose the R.Q. rose rapidly to values greater than 1.0. Similar results had been obtained by Higgins (1916) and Benedict and Carpenter (1918), and were later reported by Talbott, Coombs, Consolazio & Pecora (1938). Cathcart and Markowitz measured the blood sugar in each case and concluded that the results were not due to differences in absorption. Cori and Cori (1928) how-:ever, pointed out that this was an erroneous conclusion since blood sugar levels depend on both rates of absorp-:tion and utilization of carbohydrates.

Soskin and Levine (1952) emphasised the complex nature of the R.Q. and the multiplicity of inter-:conversions of metabolites which determine the over-all value. Werthessen (1937) trained rats to eat their day's food intake in 1 to 5 hours after which he measured their R.Q. at frequent intervals for 15 to 20 hours. In some of his experiments a normal range of R.Q. values was recorded though values as high as 1.7 and as low as 0.3 were observed. These results are said to have been confirmed by Markowitz using himself as experimental subject, (Soskin & Levine, 1952). Werthessen pointed out that controlled feeding seriously affects the animal's metabolism and this finding has been confirmed by recent workers (Tepperman, Brobeck & Long, 1943); Cohn & Joseph, 1960; Hollifield & Parson, 1962). In referring to Werthessen's results Tepperman et al. state, "analytical results obtained in controlled feeding experiments cannot be invoked as evidence in a discussion of the sugar-disposal habits of animals with normal eating habits".

In a stable animal the measured R.Q. has been shown to correspond accurately with the theoretical R.Q. of the diet and to rise and fall linearly in relation to a decrease or increase in the amount of food eaten (Dewar & Newton, 1948) That measurements of the R.Q. in human beings, under conditions which are not precisely con-:trolled, do reflect the metabolic changes, is indicated

by Table 22. The mean of a large number of R.Q. measurements obtained while the subjects were occupied in various activities, not involving heavy exercise, and at all times of the day unrelated to meals is compared with the theoretical R.Q. of their diets. The dietary intake at the time of the measurements was determined by dietary survey or prescribed in the metabolic ward of a hospital. There was no evidence that any of these subjects was not in calorie balance at the time. The experiments on the two fat women are described in detail in this thesis and the R.Q's. of the other 12 subjects were determined by the same method. The general agree-:ment between the observed and calculated R.Q. values for the quotients obtained as described in this thesis, do reflect with some accuracy the sum of the metabolic changes in the body. It therefore seems valid to use indirect calorimetry for calculating changes in body composition.

In all of the experiments on human subjects reported in this thesis it was found to be difficult to raise the R.Q. above 1.0. In the experiments of Series 1, 2 & 3 there were only 27 R.Q's. which reached 1.00 and only 3 of these were greater than 1.04, out of a total of 285 measurements. To obtain R.Q's. above 1.0 requires a considerable period of time; the mean R.Q. reached this level on the sixth day with Betty but only on the ninth day of overfeeding with Pat.

Table 22.

A comparison of the theoretical R.Q. of the food intake with the mean value of all the measurements of the R.Q. made on the subjects, when carrying out various activities and at irregular intervals throughout the day.

Subjects	No. of subjects	No. of measurements of R.Q.	Observed R.Q. (mean)	Calculated R.Q. of diet	Reference
Colliery clerks	10	210	0.84	0.86	Unpublished material obtained during study by Garry, Passmore, Warnock & Durnin (1955)
Thin male students	2	46	0.82	0.85	Passmore, Meiklejohn, Dewar & Thow, (1955 a,b)
Fat young women	2	72	0.86	0.86	

(All subjects were on diets which approximately met their calorie needs. There was no evidence of gains or losses of weight).

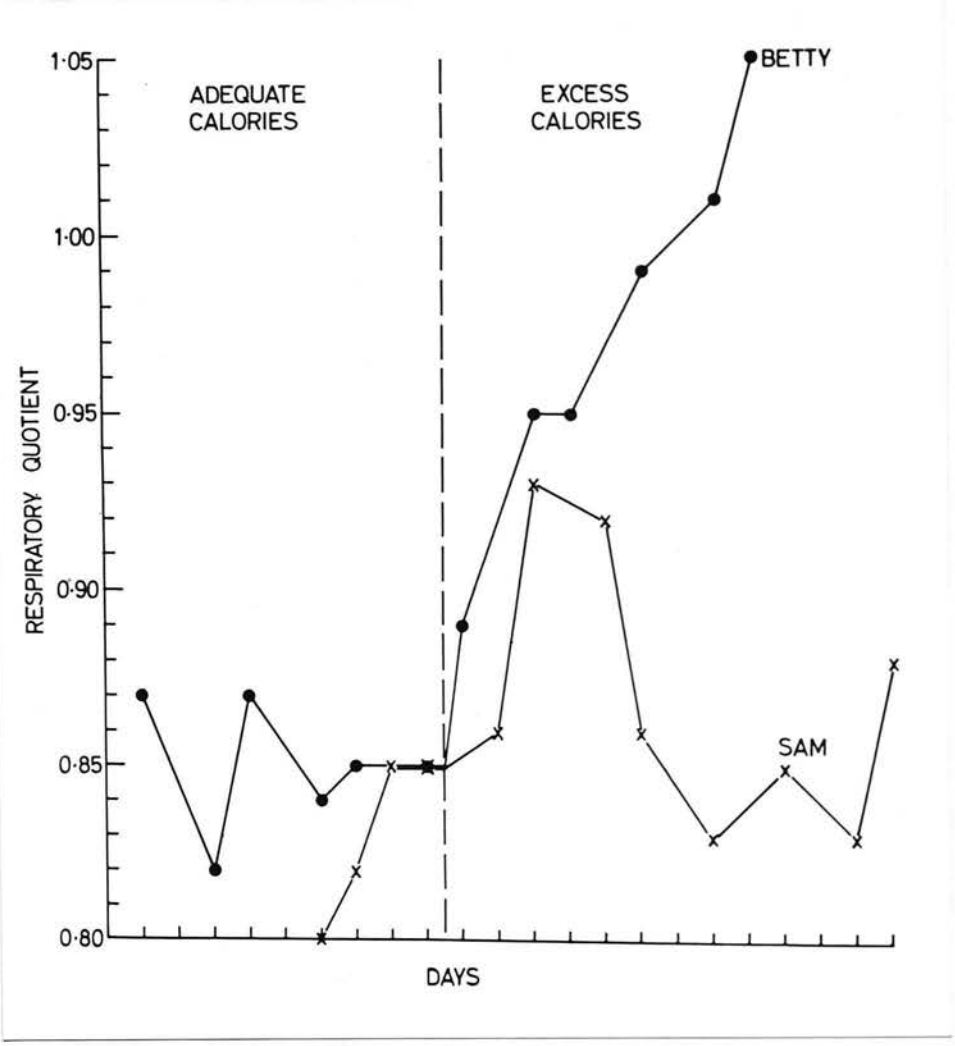
Table 23.

Respiratory quotients measured for two thin young men during a period of 4 days on a control diet which just met calorie requirements and 14 days when an excess of calories was consumed (Passmore, 1952). Each value is the mean of 6 measurements.

Adequate Calories			Excess Calories		
Day	Michael	Sam	Day	Michael	Sam
1	.83	.80	2	.85	.86
2	.82	.82	3	.88	.93
3	.80	.85	5	.88	.92
4	.80	.85	6	.85	.86
			8	.82	.83
			10	.83	.85
			12	.83	.83
			13	.83	.88

Fig. 8.

The mean daily R.Qs. of a fat woman and a thin man when an adequate diet was followed by an excess caloric intake.



The R.Q's. rose even more slowly in the experi-:ments of Passmore et al. (1955 a, b). The R.Q's. for the thin men were determined but were not published; they are presented in Table 23. The marked difference between the R.Q's. of the fat women and the thin men (Tables 39 and 23) is illustrated by Fig. 8. Thus 3 differences have been noted between the thin men (Passmore et al.; 1955 a, b) and the fat women studied here. During overfeeding the overweight women exhibited:-

(1) a more rapid gain in body weight;

(2) a greater storage of water;

(3) a more rapid rise in R.Q.

Obviously the numbers of subjects are too few for general conclusions to be drawn.

If the R.Q. is a valid index of carbohydrate metabolism (as discussed above), there are two possible explanations of the slow rise in R.Q. in all the experiments. Firstly some of the O_2 might have been used in the desaturation of fat in the following manner:-

$$-CH_2\ -CH_2\ - + O \quad = -CH{=}CH\ - + H_2O.$$

This explanation was suggested by Furnass (1960) who also failed to observe high R.Q.'s. when 4 subjects received 270 g. glucose after 4 days on a diet rich in carbohydrate. Though the desaturation of fat might be the explanation of very low R.Q's. following a high-fat diet (as suggested by Hawley, Johnson & Murlin, 1933) it

is an unlikely result of a large intake of carbohydrate since carbohydrate feeding is a basic principle in the hardening of fat in meat production. Ellis (1933) noted the following changes in the body fat of young pigs when their carbohydrate intake was increased by feeding them maize, in place of a diet based on peanuts:- the iodine number was reduced from 93 to 63, the per-:centage of saturated fatty acids increased from 19 to 33 and the percentage of linoleic acid was reduced from 23 to 9. Likewise, Longenecker (1939) observed a decrease from 84 to 78 in the percentage of unsaturated fatty acids in the depot lipids of rats when fed a diet rich in carbohydrate. Cathcart & Cuthbertson (1931) found iodine values of 69 to 85 in the liver fat of 2 overweight women but values of 121 to 134 in 7 normal subjects. All these results suggest that the fat formed from carbohydrate is likely to be saturated and they give no support to the theory proposed by Furnass (1960).

The second explanation for the slow rise in the R.Q's. is the temporary storage of carbohydrate before its conversion to fat. This store would probably be as glycogen since, in the experiments of Series 2, the blood glucose was unchanged and there was no glycosuria resulting from overfeeding.

The lack of information regarding the glycogen re-:serves of the human body was indicated in Chapter II.

In the example of the hypothetical man quoted by Soskin and Levine (1952) it was calculated that the liver contained 108 g. glycogen and the muscles 245 g. The storage capacity of the liver is obviously limited. Even if the liver glycogen rose to a level of 10% after an excess intake of carbohydrate, its storage capacity for glycogen would be less than 200 g. However, to store an extra 500 g. in muscle would require a rise in muscle glycogen of only 1.4%. The report of Hildes et al. (1949) revealed a range of glycogen content of muscle of 0.78 to 3.89% so an increase of 1.4% would not be impossible. Their subjects were convalescing hospi-:tal patients but no comparable studies have been made with healthy people receiving a carbohydrate excess. To study this aspect would require a large series of muscle biopsies since even in the limited experiments of Hildes et al. there was a difference found in the glycogen content of the two muscles sampled. ($2.20 \pm$ 0.23% for pectoralis major and $1.30 \pm$ 0.13% for gastro-:cnemius muscle). In the present investigation the experiment with rats was planned to test the hypothesis of carbohydrate storage in muscle after an excess intake.

R.Q's. and glycogen reserves in animals. In the experiments with rats described in Chapter IV the mean muscle glycogen ranged from 0.29% (mean R.Q. 0.81) to 0.64% (mean R.Q. 1.03) for groups 1 and 4 respectively, (Fig. 6). More experiments are required to establish

the upper limits of glycogen storage with an increase in both carbohydrate intake and in the length of the experiment.

Similar results have been found by other experi-:menters. Between 1925 and 1930 Cori & Cori reported many experiments on glycogen formation in rats. When rats which had been fasted for 24 h. were given glucose the mean total glycogen content increased from 140 to 595 mg./100 g. body weight in 4 h. Less than half of the stored glycogen was found in the liver (Cori & Cori, 1928). Likewise Hines & Knowlton (1935) observed an increase in the glycogen content of gastrocnemius muscle of rats after glucose feeding. Bridge (1937) measured the total glycogen in 119 rabbits which had been fasted or fed to give a range of basal R.Q's. from 0.68 to 1.03. The total glycogen content varied from 3.0 to 17.4 g./kg. net body weight. Sokal & Sarcione (1959) reported 0.64% glycogen in the muscle of rats 4 - 5 h. after receiving glucose (0.75 g./100 g. body weight) compared with 0.44% in rats fasted for 24 h.

In the liver the levels of glycogen respond rapidly to periods of feeding and starvation. For this reason it is usual to precede a study of glycogen reserves, by a starvation period. This was omitted in the present experiment since the aim was to study the effects of a carbohydrate excess, with particular emphasis on changes in muscle glycogen. The glycogen content of muscles is

less sensitive to starvation since muscles lack the phosphatase necessary to convert hexose phosphates to blood glucose. Consequently 6 h. of fasting produced no change in the level of muscle glycogen (groups 1 & 2).

Throughout the experiment the glycogen content of abdominal muscle was always higher than that of leg muscle, a difference similar to that reported by Hildes et al. (1949) in man. In the experiments with rats this difference may have been accentuated by the sampling procedure since the abdominal muscle was always dissected before the leg muscle.

No conclusive evidence regarding glycogen storage in subcutaneous fat was given by the analysis of the skin. Only in rat No. 11 which had the maximum R.Q. of 1.10, did the skin contain more than negligible amounts of glycogen.

The R.Q's. measured for the 4 groups of rats are remarkably consistent within each group. In endeavouring to increase the carbohydrate intake for group 4, the conditions were less controlled and the muscle glycogen levels were more scattered.

The relationship between the R.Q. and glycogen reserves is probably best illustrated in hibernating animals which store both glycogen and fat in large amounts. Pembrey (1902) observed R.Q's. of 1.02 to 1.39 in marmots preparing for hibernation. During hiber-:nation the R.Q's. are low but they rise during periods of arousal when glycogen is being used for energy needs,

(Kayser, 1961).

In comparing the results of animal experiments with experiments in man Dreyfus, Schapira, Schapira & Demos (1956) reported that the activity of phos-:phorylase in human muscle is only 30% of the levels found in rats and rabbits. If this observation has general application it would suggest a greater capacity for glycogen storage in human muscle.

Glycogen storage in man. From the evidence presented in this thesis it appears than an intake of carbohydrate in excess of the energy needs of human subjects, is followed by a temporary storage of glycogen prior to its conversion to fat. Although this conslusion is based on indirect evidence with human subjects, no reason can be found for not accepting the classical interpretation of R.Q. values if proper care is exercised in their measurement, and the number of measurements is sufficiently large. The hypothesis is supported by experiments on rats and by the range of levels of glycogen in muscle reported by Hildes et al. (1949). It therefore seems likely that the capacity for carbo-:hydrate storage in man is greater than is usually assumed, and that it should not be ignored when discussing changes in body composition. However it is also realised that the capacity for carbohydrate storage in the human body is very much smaller than the tremen-:dous capacity for the storage of fat.

SOURCES OF ERROR IN THE RESULTS

Table 17 gives the mean daily intake, output and utilization of energy, protein, fat, carbohydrate and water and the change in body weight during the control period of 9 days for Betty and Pat. It contains results of the typical analytical methods reported in this thesis and will be used as an example for discussing the possible sources of error.

1. The weight balance and the water balance. The body weights are the most accurate of all the measurements reported. The metabolic balance used in these experiments has an accuracy of ± 0.001%. From the change in body weight and the weights of food, faeces and urine a weight balance was drawn up each day, and the water balance is directly related to it.

2. The diet. In the diet to be analysed, each food was weighed out at the same time as the 2 diets which were to be eaten. The drinking water was kept in bottles distinctly labelled for each subject and the volumes noted at least twice daily. Some errors occur in the chemical analyses of the diet but an independent check of the protein, carbohydrate and fat content is given by the bomb calorimetry. The carbohydrate content was estimated after digestion of the food and standard with takadiastase and it has been assumed that this is the total available carbohydrate.

3. The faeces. The errors in the collection, sampling

and analysis of faeces are greater than for the diet but the faeces contributes a relatively small proportion to the energy and weight balances; the faecal solids were 23 to 41 g. compared with 615 g. food solids. Here, too the bomb calorimetry gives independent con-:firmation of the results.

4. The urine. Great care was taken to collect the total urine in all the experiments and its volume and specific gravity were measured daily. The urine solids were not determined but were calculated by the formula of Trapp (1850). Similarly for the urinary calories the assumption that the energy value is equivalent to 7.9 kcal./g. has been used (Atwater & Bryant, 1900). Any errors in these assumptions will have an insignificant effect on the total balance since the solids represent less than 5% of the total urinary weight.

5. Losses from the skin and lungs. It is known that there are small losses of N from the skin though these are difficult to determine. The daily loss of 1 g. protein in this way was assumed from the direct mea-:surements reported by Darke (1960). The evaporative water loss could be subject to error since it is based on the insensible weight loss calculated by difference from the daily weight balance. However, the constancy of the heat lost by this means, as postulated by Newburgh et al. (1937) was confirmed throughout this experiment.

6. The metabolic mixture. Calculation of the metabolic

mixture is based on the assumption that the sampling of the expired air and the record of activities is fully representative of the energy expenditure for 24 h. A more accurate measurement of energy expenditure can be obtained by direct calorimetry but it is impossible to simulate normal living conditions with a subject in a metabolic chamber. Only indirect calorimetry is suitable for such measurements. The samples of expired air were collected at irregular intervals throughout the day and night, in an attempt to get typical results for representative activities for each period. The samples were analysed in duplicate and whenever more than one apparatus was used tests were carried out to ensure comparable results. The Max-Planck respirometer was calibrated against the same gas meter as used in conjunction with the Douglas Bag. The amounts of protein metabolised are based on the direct determination of urinary N and are subject to the least error of the components of the metabolic mixture. The assumption that protein contains 16% N enters into all the figures in the protein balance in Table 17.

The only other analyses carried out, in addition to those discussed above are the estimations of ketones, N.E.F.A., glucose and glycogen. In all cases, blank and standard solutions were included in each experiment; each method was given preliminary checking with recovery experiments.

7. The Balance. The fact that the 2 subjects lost a little weight during the control period though their caloric balance was calculated as +60, is probably an indication of the size of the overall error in the measurements. An error of 100 kcal. in the total balance would represent 3% of the caloric intake and up to 5% in the amounts of carbohydrate and fat metabolised. It would appear unlikely that the overall error is greater than 5%.

SUMMARY.

1. In 19 experiments of short duration (arranged in 3 series) it was found difficult to raise the R.Q. in man above 1.0 after an excess intake of carbohydrate.
2. In Series 1, 8 subjects received 4 meals supplying a total of 399-505 g. carbohydrate and the maximum mean R.Q. was 0.94 in a 14 h. period.
3. In Series 2, 9 men received 597-1172g. carbohydrate in 5 meals. During 24 h. a maximum mean R.Q. of 0.99 was reached at 2.0 a.m. The full water balance was determined.
4. The period of study was extended to 8 days in Series 3 when the effect of a mid-day meal rich in carbo-:hydrate was followed for 6h. each day, with 2 sub-:jects. The meal was superimposed on a normal diet for 2 days, a diet low in fat for 4 days and a diet containing excess carbohydrate for 2 days. Even on the final day the net conversion of carbohydrate to fat was negligible.
5. The effects of overeating for a longer period were investigated with 2 overweight women while in hospit-:al for 23 days. After 9 days on a control diet, they received an excess intake for 9 days and were underfed for 5 days. During the overfeeding period they received 7290 and 10440 kcal in excess of their energy expenditure and gained respectively 2.86 and 2.58 kg. in 9 days. During the 5 days of under-

:feeding they lost 6.22 and 5.63 kg. with a negative calorie balance of 12100 and 13850 kcal. re-:spectively. The changes in body composition for each period were calculated.

6. The interpretation of R.Q.'s and their validity as an index of the metabolic mixture, is discussed.

7. The slow rise in the R.Q. and the small net con-:version of carbohydrate to fat in these experiments suggests a temporary storage of carbohydrate before its conversion to fat. This hypothesis was tested by an experiment with rats. With a mean R.Q. of 0.81 the glycogen content of rat muscle was 0.29% while with an R.Q. of 1.03 the muscle tissue con-:tained 0.64% glycogen. In both man and rats there appears to be a delay in the net conversion of carbohydrate to fat following an excess intake of carbohydrate.

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A P P E N D I X.

Tables 24 - 33.	Experiments of short duration on human subjects.
Tables 34 - 41.	Longer experiment on human subjects.
Tables 42, 43.	Experiment on rats.

Table 24.

A The sex, heights and weights of 8 subjects who took part for 14 h. in the experiments of Series I during which they were overfed with meals rich in carbohydrate.

Subject	Date	Sex	Height cm.	Weight kg.
Dav.	10.8.61	F	166	62
Gil.	15.8.61	F	169	55
Nas.	23.6.61	M	177	70
Pas.	6.6.61	M	183	65
Shi.	27.6.61	M	180	79
Swi.	13.6.61	F	163	65
Tho.	15.6.61	F	178	68
Whi.	17.8.61	F	165	51

Table 24.

B. The initial and final body weights, the intake of food and fluid, the output of urine and faeces and the insensible weight losses (all in g.) of 9 men during 24 h. of overfeeding with meals rich in carbohydrate.

Subjects	Date	Body weight		Intake		Output		
		Initial	Final	Food eaten	Water drunk	Urine	Faeces	Insen-:sible weight loss
Bow.	17.10.61	60.892	60.565	1160	1360	1241	-	1606
Har.	20.10.61	59.460	59.954	1819	1530	795	-	2060
Nas.	26. 9.61	67.079	68.562	1476	2210	981	130	1092
New.	12.10.61	62.446	62.457	1525	1020	901	165	1468
Nim.	13.10.61	66.348	68.077	1884	2160	867	-	1508
Pas.	19. 9.61	64.354	65.857	1714	2090	956	73	1269
Pic.	23.10.61	90.269	90.888	1820	3230	1666	-	2765
Ste.	11.10.61	55.693	56.048	1500	2720	2391	-	1474
Wil.	19.10.61	69.188	70.491	2097	2750	843	390	2311

Table 24.

C. The heights and weights of the 2 subjects who took part in the experiments of Series 3.

Subject	Basic Diet	Day	Date	Body Weight (Kg.)	Body Weight (Kg.)
Pas. (Male) (Height) (183 cm)	Mixed	2	8.12.61	64.9	+ 0.3 (6 days)
	Low	5	11.12.61	65.4	
	Fat	6	12.12.61	65.1	
	Excess Carbo-hydrate	7	13.12.61	65.2	
		8	14.12.61	65.2	
Swi. (Female) (Height) (163 cm)	Mixed	1	30.11.61	65.2	+ 2.8 (7 days)
		2	1.12.61	65.4	
	Low	5	4.12.61	65.6	
	Fat	6	5.12.61	65.4	
	Excess Carbo-hydrate	7	6.12.61	66.3	
		8	7.12.61	68.0	
	Normal	9	8.12.61	66.0	- 3.0 (3 days)
		10	9.12.61	64.8	
		11	10.12.61	65.0	

Table 25.

The timetables for the experiments of Series 1, 2 and 3 in which some effects of meals rich in carbohydrate were studied. In between the meals the subjects sat and read except for short periods of walking on a treadmill.

A. Series 1. 14 h.

Period	Subjects weighed	Expired air samples collected during rest		Walking on treadmill	Meal
0	8.00	9.00		9.20 - 9.40	9.45
1		11.00	12.00	12.20 - 12.40	12.45
2		14.00	15.00	15.20 - 15.40	15.45
3		17.00	18.00	18.20 - 18.40	18.45
4		20.00	21.00	21.20 - 21.40	-

B. Series 2. 24 h.

Period	Subjects weighed	Blood sample collected	Expired air samples collected during rest	Walking on treadmill	Meal
0	9.00	9.10	9.50	-	10.00
1			12.15	12.40 - 12.50	13.00
2			15.15	15.40 - 15.50	16.00
3			18.15	18.40 - 18.50	19.00
4			21.15	21.40 - 21.50	22.00
5			Between 0.00 and 1.00	-	-
6			B etween 3.00 and 5.00	-	-
7			6.30	-	-
8			8.00	8.30 - 8.40	-
	9.00	9.10			

C. Series 3. 6 h. daily during 8 days.

Meal	12.00
Expired air samples collected during rest	13.30, 14.00, 14.30, 15.00, 15.30, 16.00, 16.30, 17.00.

Table 26.

The Meals.

The figures obtained by analysis for the total intake of water, protein, fat and carbohydrate (all in g) in meals consisting of white bread, apricot jam, tinned peaches, tea and sugar. The calculated net calorie values of the meals are also given.

A. Series I. 4 meals.

Subject	Water in food and drink	Protein	Fat	Carbo-hydrate	Calories kcal
Dav.	1745	40	1	399	1760
Gil.	1039	40	1	417	1820
Nas.	1034	38	1	331	1480
Pas.	1632	53	2	422	1900
Shi.	1111	49	2	505	2220
Swi.	1800	50	2	461	2050
Tho.	1785	49	2	455	2020
Whi.	1039	39	1	374	1660
MEAN	1400	45	2	421	1870

B. Series 2. 5 meals.

Subject	Water in food and drink	Protein	Fat	Carbo-hydrate	Calories kcal
Bow	1832	52	2	597	2610
Har.	2173	85	3	1022	4460
Nas.	2810	87	3	750	3380
New	1641	86	3	774	3470
Nim.	2868	96	3	1018	4480
Pas.	2779	96	3	874	3910
Pic.	3918	89	3	981	4310
Ste.	3324	80	3	768	3420
Wil.	3500	101	3	1172	5120
MEAN	2760	86	3	884	3910

C. Series 3. 1 experimental meal each day.

Subject	Water in food and drink	Protein	Fat	Carbo-hydrate	Calories kcal
Pas.	979	24	1	204	920
Swi.	909	24	1	204	920

Table 27.

The oxygen consumption (in ml/min) determined by analysis of expired air collected while subjects were sitting at rest.

A. Series 1. 14 h.

Subject	PERIODS									MEAN
	0	1		2		3		4		
Dav.	203	250	229	233	215	249	217	253	224	230
Gil.	214	235	227	233	234	247	257	254	221	236
Nas.	221	262	248	244	240	263	225	240	227	241
Pas.	210	272	255	264	246	281	252	317	247	260
Shi.	265	289	294	283	286	297	-	285	279	285
Swi.	181	177	222	274	208	220	216	229	210	215
Tho.	226	285	266	303	263	291	275	280	256	281
Whi.	208	224	219	241	214	234	209	191	207	216
MEAN	216	249	245	259	260	248	242	256	234	246

B. Series 2. 24 h.

Subject	PERIODS									MEAN
	0	1	2	3	4	5	6	7	8	
Bow.	-	285	306	346	307	289	273	251	243	288
Har.	321	311	313	336	336	369	-	288	297	321
Nas.	210	249	242	251	253	242	212	210	218	236
New.	290	307	379	-	367	347	334	283	-	336
Nim.	248	317	307	-	325	306	317	295	-	306
Pas.	254	280	296	304	320	340	230	224	243	280
Pic.	404	332	360	306	423	397	283	328	-	347
Ste.	241	297	316	-	286	298	-	199	-	273
Wil.	292	353	326	370	357	337	321	322	279	333
MEAN	283	303	316	319	330	325	281	267	256	302

C. Series 3. 6 hours daily during 8 days.

Periods		1	2	3	4	5	6	7	8	
Subjects	Day									MEAN
Pas.	5	313	327	340	324	-	278	275	265	303
	6	305	296	308	300	274	267	263	267	285
	7	311	332	319	314	287	265	-	248	297
	8	333	329	-	266	290	282	268	234	286
Swi.	1	239	242	232	241	229	240	229	235	236
	2	238	239	240	239	239	211	214	220	230
	5	247	243	247	248	254	243	229	238	244
	6	231	245	236	236	237	243	226	212	233
	7	251	262	250	252	234	237	222	199	239
	8	282	284	280	274	266	269	252	253	270

Table 28.

The oxygen consumption (in ml/min) determined by analysis of expired air collected while the subjects were walking on a treadmill.

A. Series 1. Each value is the mean of results from 2 air samples collected during each walk. The subjects walked at 4 m.p.h. except those marked * who walked at 3.3 m.p.h.

Subject	Periods					
	0	1	2	3	4	Mean
Dav.*	881	845	805	812	811	831
Gil.*	802	760	752	754	764	766
Nas.	-	-	1285	1253	1263	1267
Pas.	1128	1103	1123	1133	1146	1127
Shi.	1457	1429	1402	1405	1411	1421
Swi.	1152	1145	1130	1145	1125	1139
Tho.	1073	1078	1104	1144	1129	1106
Whi.*	800	763	745	747	766	764

B. Series 2. The subjects walked at 4 m.p.h. except Nas. who walked at 3.5 m.p.h.

Subject	Periods					
	1	2	3	4	5	Mean
Bow.	-	-	-	1465	-	
Har.	1347	-	1323	-	1290	1311
Nas.	1101	1063	1067	967	1023	1044
New.	1347	1443	1398	-	-	1396
Nim.	1261	1322	1452	-	-	1345
Pas.	1169	1259	1222	1268	1249	1230
Pic.	2062	2025	2000	2034	1725	1969
Ste.	1232	1152	1220	1157	1038	1160
Wil.	-	-	-	-	-	

Table 29.

Respiratory quotients determined by analysis of expired air collected while subjects were sitting at rest.

A. Series 1. 14 h.

Subject	Period									
	0	1		2		3		4		Mean
Dav.	0.82	0.86	0.91	0.90	0.93	0.93	0.92	0.92	0.93	0.90
Gil.	0.83	0.89	0.88	0.88	0.95	0.95	0.97	0.97	0.95	0.92
Nas.	0.85	0.89	0.92	0.90	0.93	0.93	0.92	0.92	0.90	0.91
Pas.	0.82	0.86	0.86	0.88	0.85	0.85	0.90	0.84	0.89	0.86
Shi.	0.77	0.89	0.81	0.88	0.90	0.91	-	0.92	0.96	0.88
Swi.	0.83	0.90	0.91	0.91	0.93	0.91	0.92	0.91	0.93	0.91
Tho.	0.80	0.91	0.92	0.98	0.92	0.95	0.93	1.02	0.97	0.93
Whi.	-	0.96	0.85	1.00	1.01	0.96	0.96	0.98	0.97	0.96
Mean	0.82	0.90	0.88	0.92	0.93	0.92	0.93	0.94	0.94	0.90

B. Series 2. 24 h.

Subject	Period									
	0	1	2	3	4	5	6	7	8	Mean
Bow.	0.73	0.78	0.75	0.84	0.90	0.93	0.87	0.87	0.78	0.84
Har.	0.78	0.93	0.99	0.97	1.01	1.02	-	0.90	0.77	0.94
Nas.	0.84	0.89	0.91	0.89	0.92	0.95	0.93	0.97	0.90	0.91
New.	0.78	0.87	0.85	0.91	0.88	0.85	0.95	0.95	0.91	0.90
Nim.	-	0.93	0.81	-	1.04	1.04	1.04	0.98	-	0.98
Pas.	0.77	0.86	0.86	0.87	0.88	0.91	1.04	0.96	0.88	0.91
Pic.	0.84	0.87	1.01	0.89	0.96	0.92	1.00	0.98	-	0.95
Ste.	0.80	0.94	0.94	-	1.06	1.00	1.01	-	0.91	0.98
Wil	0.86	0.93	0.94	1.05	1.00	1.03	1.09	0.96	0.98	1.00
Mean	0.80	0.88	0.90	0.92	0.96	0.96	0.99	0.95	0.88	0.91

C. Series 3. 6 h. daily during 8 days.

Periods		1	2	3	4	5	6	7	8	Mean
Subject	Day									
Pas.	5	0.96	0.95	0.93	0.93	-	0.94	0.92	0.94	0.94
	6	0.90	0.92	0.93	0.96	0.97	0.95	0.97	0.97	0.95
	7	0.97	0.95	0.96	0.94	0.94	0.94	-	0.92	0.95
	8	0.98	0.97	-	0.96	0.96	0.94	0.98	0.95	0.96
Swi.	1	0.86	0.87	0.86	0.90	0.88	0.91	0.91	0.89	0.89
	2	0.88	0.88	0.90	0.91	0.92	0.90	0.90	0.92	0.90
	5	0.93	0.90	0.90	0.92	0.93	0.93	0.91	0.95	0.92
	6	0.92	0.91	0.90	0.93	0.94	0.93	0.91	0.90	0.92
	7	0.96	0.94	0.94	0.93	0.93	0.94	0.95	0.91	0.94
	8	1.03	0.99	1.00	0.99	0.99	1.00	1.00	1.01	1.00

Table 30.

Respiratory quotients determined by analysis of expired air collected while subjects were walking on a treadmill.

A. Series 1. The subjects walked at 4 m.p.h. except those marked * who walked at 3.3 m.p.h.

Subject	Period					
	0	1	2	3	4	Mean
Dav.*	0.87	0.96	0.96	0.96	0.97	0.94
Gil.*	0.83	0.91	0.95	0.96	0.95	0.92
Nas.	-	-	0.97	0.95	0.96	0.96
Pas.	0.85	0.93	0.90	0.92	0.92	0.90
Shi.	0.87	0.92	0.94	0.95	0.95	0.93
Swi.	0.85	0.95	0.95	0.96	0.98	0.94
Tho.	0.85	0.92	0.96	0.92	0.97	0.91
Whi.*	0.86	0.93	0.96	0.95	0.99	0.94
Mean	0.85	0.93	0.95	0.95	0.96	0.93

B. Series 2. The subjects walked at 4 m.p.h. except Nas. who walked at 3.5 m.p.h.

Subject	Period					
	1	2	3	4	5	Mean
Bow.	0.98	0.99	0.97	0.94	-	0.97
Har.	0.90	0.96	1.00	1.01	-	0.98
Nas.	0.89	0.93	0.92	0.92	0.87	0.91
New.	1.01	0.93	0.90	-	-	0.95
Nim.	0.99	0.95	0.92	-	-	0.95
Pas.	1.01	0.96	0.96	0.97	0.93	0.96
Pic.	0.89	0.90	0.95	0.94	-	0.92
Ste.	0.94	0.95	0.93	-	0.93	0.94
Wil.	-	-	-	-	-	
Mean	0.95	0.95	0.95	0.96	0.91	0.95

Table 31.

The time spent walking, sitting and up and about, the total volume of oxygen utilized and the total output of carbon dioxide and urinary nitrogen. The figures for O_2 and CO_2 were calculated from the mean values in Tables 27 - 30, and from the times given here.

A. Series 1. 14 h.

Subjects	Time spent (min)				Oxygen Utilization l.	Carbon Dioxide Output l.	Urinary Nitrogen g.
	Walking	Sitting	Up and About	Total			
Dav.	108	697	35	840	264.0	241.3	5.9
Gil.	108	697	35	840	261.2	240.3	5.7
Nas.	101	705	34	840	311.5	290.0	5.8
Pas.	100	700	40	840	310.7	271.8	6.8
Shi.	102	708	30	840	358.7	323.1	6.9
Swi.	115	665	60	840	298.0	275.3	5.5
Tho.	104	645	91	840	332.6	306.7	5.9
Whi.	102	703	35	840	243.7	232.2	4.0
Mean	104	690	45	840	297.6	272.6	5.8

B. Series 2. 24 h.

	Time spent (min)			
	Walking	Sitting	Up and About	Total
All Subjects	50	1330	60	1440

Subject	Oxygen Utilization l.	Carbon Dioxide Output l.	Urinary Nitrogen g.
Bow.	492.3	424.1	9.3
Har.	528.5	499.8	14.0
Nas.	397.7	361.9	10.6
New.	552.7	501.6	11.3
Nim.	510.3	497.5	11.2
Pas.	463.5	423.8	16.0
Pic.	602.0	568.5	15.3
Ste.	451.1	438.8	11.6
Wil.	547.5	547.5	9.2
Mean	505.1	473.7	12.1

C. Series 3.

The subjects sat in an easy chair for the whole 6 h. period each day.

Subject	Day	Oxygen Utilization l.	Carbon Dioxide Output l.	Urinary Nitrogen g.
Pas.	5	109.1	102.6	3.6
	6	102.6	97.6	3.4
	7	106.9	101.5	2.2
	8	103.0	99.0	2.4
Swi.	1	85.0	75.6	3.0
	2	82.8	74.5	2.7
	5	87.8	80.6	2.7
	6	83.9	77.0	2.0
	7	86.0	81.0	2.8
	8	97.2	97.2	2.5

Table 32.

The Metabolic Mixture.

The weights of protein, fat and carbohydrate metabolised, calculated from the total utilization of oxygen and output of carbon dioxide and urinary nitrogen using the assumptions of Zuntz (1897). The calculated calorie value of the metabolic mixtures and the percentage of the calories derived from protein, fat and carbohydrate are also given.

A. Series 1. 14 h.

Subject	Metabolic Mixture				Calories Derived From		
	Protein g.	Fat g.	Carbo-hydrate g.	Calories kcal.	Protein %	Fat %	Carbo-hydrate %
Dav.	37	27	211	1270	12	20	68
Gil.	36	24	215	1250	12	18	70
Nas.	36	25	273	1500	10	15	75
Pas.	43	53	198	1480	12	33	55
Shi.	43	47	270	1720	10	26	64
Swi.	34	28	253	1440	10	18	72
Tho.	37	32	281	1600	9	19	72
Whi.	25	12	237	1190	9	9	82
MEAN	36	31	242	1430	10	20	70

B. Series 2. 24 h.

Subject	Metabolic Mixture				Calories derived From		
	Protein g.	Fat g.	Carbo-hydrate g.	Calories kcal.	Protein %	Fat %	Carbo-hydrate %
Bow.	58	97	291	2360	10	40	50
Har.	88	22	485	2580	14	8	78
Nas.	66	40	306	1920	14	20	66
New.	71	64	430	2680	11	21	68
Nim.	70	0	536	2510	12	0	89
Pas.	100	36	356	2240	19	15	66
Pic.	96	26	553	2930	14	8	78
Ste.	73	-2	466	2210	14	-1	87
Wil.	58	-18	640	2710	9	-6	97
MEAN	76	30	452	2460	13	12	75

C. Series 3. 6 h.

6

Subject	Day	Metabolic Mixture				Calories Derived From		
		Protein g.	Fat g.	Carbo-hydrate g.	Calories kcal.	Protein %	Fat %	Carbo-hydrate %
Pas.	5	23	4	96	520	18	7	75
	6	21	2	95	490	18	3	79
	7	14	5	102	490	12	3	85
	8	15	2	102	500	12	4	84
Swi.	1	19	10	56	400	19	24	57
	2	17	9	59	390	18	20	62
	5	17	7	70	420	16	16	68
	6	13	8	68	400	13	18	69
	7	18	3	76	410	17	7	76
	8	16	-5	111	470	14	-10	96

Table 33.

The Blood.

A. Series 2. The figures obtained by analysis for the content of glucose, non-esterified fatty acids (N.E.F.A.) and total ketones (all in mM./l.) of blood plasma at the start and end of each experiment in Series 2.

	Glucose		N.E.F.A.		Ketones	
	Start	End	Start	End	Start	End
Bow.	6.06	5.23	1.13	0.89	(1.42)	(0.29)
Har.	4.67	5.17	0.95	0.33	0.20	0.09
Nas.	4.34	4.28	0.78	0.49	0.18	0.09
New.	4.56	5.12	0.58	0.52	0.24	0.06
Nim.	4.45	4.95	0.57	0.54	0.14	0.08
Pas.	4.39	4.56	1.02	0.43	0.23	0.09
Pic.	4.17	4.34	0.54	0.61	0.13	0.17
Ste.	4.95	4.23	0.45	0.38	0.26	0.22
Wil.	3.89	4.78	0.74	0.70	0.21	0.16
Mean	4.61	4.74	0.75	0.54	0.20*	0.12*

(* Mean ketones omitting Bow)

B. Series 3. The figures obtained by analysis for the glucose content (in mM/l) of blood plasma at the start and end of the experiemnts of Series 3.

Basic Diet	Day	Pas.		Swi.	
		Start	End	Start	End
Mixed	1	-	-	4.0	3.5
	2	-	-	4.2	5.7
Low Fat	5	4.7	4.4	4.0	6.2
	6	4.7	4.7	4.8	5.9
Excess Carbohydrate	7	4.4	6.2	4.3	3.9
	8	4.2	5.7	6.9	4.9

Table 34.

The body weights, the intake of fluid and food, the output of urine and faeces and the insensible weight losses (all in g.) measured daily for each subject during 9 days on a control diet, 9 days of overfeeding and 5 days of underfeeding.

A. Betty.

		INTAKE			OUTPUT		
	Day	Body weight	Water drunk	Food eaten	Urine	Faeces	Insensible weight loss
CONTROL	1	91934	2906	1967	3830	214	1254
	2	91504	2310	1952	2280	153	1487
	3	91842	2150	1927	3510	205	1358
	4	90850	1500	2819	2300	-	1538
	5	91335	1968	2847	2591	272	1735
	6	91552	1950	2912	3038	-	1581
	7	91795	2110	2811	3090	173	1469
	8	91984	2250	2744	3245	207	1710
	9	91816	2160	2761	3155	227	1567
OVERFEEDING	10	91787	2010	3131	3022	356	1618
	11	91932	1950	3021	2662	-	1354
	12	92887	2000	3111	2989	172	1760
	13	93077	2500	3089	3144	232	1458
	14	93832	2090	3024	2824	356	1672
	15	94094	2000	2938	2821	138	1616
	16	94457	1450	3067	2790	167	1622
	17	94395	1950	3203	3309	114	1653
	18	94472	1950	3159	3359	196	1373
UNDERFEEDING	19	94653	2940	-	4073	-	1522
	20	92000	2590	296	3293	-	1189
	21	90404	1956	666	2138	82	1015
	22	89791	2132	673	2616	-	889
	23	89091	1834	719	1964	79	1164
	24	88437	-	-	-	-	-

B. Pat.

		INTAKE			OUTPUT		
	Day	Body weight	Water drunk	Food eaten	Urine	Faeces	Insensible weight loss
CONTROL	1	92764	898	1812	1461	126	1250
	2	92637	797	1952	1334	-	1504
	3	92548	878	1927	1720	122	1397
	4	92113	660	2819	1284	-	1688
	5	92620	832	2847	1816	148	1574
	6	92761	836	2912	2168	113	1644
	7	92584	1000	2811	2270	92	1531
	8	92502	1000	2744	2150	-	1461
	9	92635	595	2761	1853	171	1902
OVERFEEDING	10	92065	1040	3061	1879	-	1392
	11	92895	608	3106	1731	266	1431
	12	93181	820	3146	1639	224	1675
	13	93609	887	3162	2035	45	1522
	14	94056	671	3199	1746	89	1564
	15	94527	623	3001	1697	120	2144
	16	94190	488	3282	1623	-	1692
	17	94645	776	3251	1888	91	1871
	18	94822	480	3119	1875	118	1776
UNDERFEEDING	19	94652	1454	-	2748	-	1307
	20	92051	1376	296	1327	117	969
	21	91310	1074	666	1427	102	1128
	22	90393	1467	673	1761	-	1040
	23	89732	1164	719	1059	98	1435
	24	89023	-	-	-	-	-

Table 35

The Diets.

The figures obtained by analysis for the intake of water, solids, protein, fat, carbohydrate (as poly-:saccharide) and ash (all in g./day), and also for the caloric values are given. The figures given for roughage were obtained by difference. The gross calorie values of the diets calculated from their content of pro-:tein, fat and carbohydrate are also included.

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
Water drunk	2145	816	1987	710	2290	1307
Water in food	1911	1911	2152	2162	404	404
Solids	615	615	927	981	67	67
Protein	131	131	145	146	14	14
Fat	147	147	91	91	11	11
Carbohydrate	297	297	645	698	35	35
Roughage	12	12	15	15	5	5
Ash	28	28	31	31	2	2
Calories (kcal)						
- bomb	3390	3390	4370	4580	350	350
- calculated	3390	3390	4380	4600	350	350

Table 36.

The Faeces.

The figures obtained by analysis for the water, solids, protein, fat and ash content of the faeces (all in g./day) and for the calorie value are given. The figures given for roughage were obtained by difference. The gross calorie values of the faeces calculated from their content of protein, fat and roughage are also included.

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
Weight	161	86	192	106	32	63
Water	120	63	141	78	22	48
Solids	41	23	51	28	10	15
Protein	12	7	18	10	4	6
Fat	10	3	11	3	2	2
Roughage	12	8	15	10	3	5
Ash	7	5	7	5	1	2
Calories (kcal)						
- bomb	240	110	280	130	60	80
- calculation	210	100	270	130	50	70

Table 37.

The Urine

The weight of urine and its content of water, solids and nitrogen (all in g./day). The calorie values were calculated as 7.9 kcal/g. urinary nitrogen. (Atwater and Benedict, 1900).

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
Weight	3004	1784	2988	1788	2817	1664
Water	2949	1723	2940	1736	2794	1639
Solids	55	61	48	52	23	25
Nitrogen	18.7	18.8	17.6	17.4	10.9	12.5
Calories (kcal)	150	150	140	140	90	100

Table 38.

The oxygen consumption (in ml/min) determined by analysis of expired air collected during representative activities at irregular intervals throughout the day and night. The basal oxygen consumption was measured at the end of each 24 h. period.

A. Betty

	Day	Sitting		Walking	In bed		Basal
		1	2		1	2	
CONTROL	1	311	309	1225	288	258	286
	2						282
	3	325	322	1239	290	243	280
	4	350	331	1139	290	259	283
	5						261
	6	285	319	1039	237	284	240
	7	347	323	1112	305	208	257
	8						269
	9	312	304	1143	278	-	283
OVER-:FEEDING	10	362	324	1064	241	288	265
	11						240
	12	340	315	1155	361	306	-
	13	331	326	1147	347	317	273
	14						260
	15	303	347	1210	321	301	272
	16						290
	17	311	350	1286	268	321	265
	18	349	346	1327	307	301	294
UNDER-FEEDING	19	292	283	996	268	-	278
	20	264	-	-	280	-	283
	21	289	313	-	321	-	277
	22	272	-	990	297	-	253
	23	234	-	1124	283	-	271

B. Pat

	Day	Sitting		Walking	In bed		Basal
		1	2		1	2	
CONTROL	1	340	317	1087	333	283	277
	2						261
	3	312	319	1209	318	266	273
	4	337	304	1051	304	276	270
	5						278
	6	274	313	1507	331	246	278
	7	357	347	1149	257	241	234
	8						286
	9	332	340	1095	311	270	283
OVER-:FEEDING	10	345	322	1040	307	272	269
	11						282
	12	308	361	1280	356	299	-
	13	337	315	1047	358	312	273
	14						274
	15	335	362	1232	332	298	268
	16						275
	17	301	325	1218	296	259	303
	18	350	360	1338	235	304	282
UNDER-:FEEDING	19	310	300	1070	300	-	296
	20	318	-	1034	359	-	318
	21	304	325	-	297	-	284
	22	274	-	1015	-	-	283
	23	265	-	1220	313	-	312

Table 39.

Respiratory quotients determined by analysis of expired air collected during representative activities at irregular intervals throughout the day and night.

A. Betty

	Day	Sitting 1	Sitting 2	Walking	In Bed 1	In Bed 2	Basal
CONTROL	1	.91	.91	.83	.87	.86	.82
	2						.82
	3	.78	.83	.85	.83	.84	.80
	4	.82	.96	.81	.86	.91	.85
	5						.89
	6	.88	.83	.86	.73	.88	.83
	7	.93	.88	.83	.79	.82	.87
	8						.87
	9	.82	.89	.81	.94	-	.81
OVERFEEDING	10	.84	.92	.89	.86	.99	.87
	11						.89
	12	.92	.92	.92	1.05	1.02	-
	13	.96	.93	.90	.95	1.02	.94
	14						.94
	15	.96	1.09	.90	1.14	1.01	.89
	16						.92
	17	.96	1.00	.91	1.06	1.08	1.04
	18	.97	1.08	.95	1.05	1.24	1.01
UNDERFEEDING	19	1.19	1.18	.80	.87	-	.83
	20	.88	-	-	.77	-	.78
	21	.94	.84	-	.85	-	.85
	22	.87	-	.78	.77	-	.78
	23	.76	-	.78	.78	-	.76

B. Pat

	Day	Sitting 1	Sitting 2	Walking	In Bed 1	In Bed 2	Basal
CONTROL	1	.86	.82	.81	.83	.81	.86
	2						.80
	3	.98	.81	.75	-	-	-
	4	.84	.98	.80	.94	.85	.86
	5						.91
	6	.84	.89	.81	.88	.86	.88
	7	.92	.91	.83	.92	.85	.86
	8						.88
	9	.87	.86	.88	.79	.85	.85
OVERFEEDING	10	.88	.95	.90	.89	.88	.86
	11						.89
	12	.95	.93	.92	1.01	.93	-
	13	.92	.88	.93	.94	.94	.94
	14						.98
	15	.96	.90	.88	1.01	.93	.90
	16						.91
	17	.99	.99	.97	1.04	1.00	.99
	18	1.02	1.00	.92	1.29	1.00	.94
UNDERFEEDING	19	1.06	.97	.84	.74	-	.73
	20	.91	-	.74	.87	-	.80
	21	.79	.80	-	.75	-	.78
	22	.89	-	.79	.79	-	.75
	23	.81	-	.73	.74	-	.72

Table 40.

DAILY ACTIVITIES

Mean values for the time (in min./day) spent in bed and in sitting, walking and up and about, obtained from a diary record.

Time spent	Control		Overfeeding		Underfeeding	
	Betty	Pat	Betty	Pat	Betty	Pat
In bed	514	512	519	516	605	587
Sitting	611	597	603	609	565	551
Walking	172	170	160	159	165	196
Up and about	143	161	158	156	105	106
Total	1440	1440	1440	1440	1440	1440

Table 41.

The Metabolic Mixtures.

The total oxygen utilization and carbon dioxide output per day were determined from the figures given in Tables 38 and 39 using these totals and the output of urinary nitrogen, the metabolic mixtures were calculated using the assumptions of Zuntz (1897).

	CONTROL		OVERFEEDING		UNDERFEEDING	
	Betty	Pat	Betty	Pat	Betty	Pat
Total oxygen (l)	622.6	642.7	648.5	649.0	552.5	621.0
Total carbon dioxide (l)	527.9	545.6	614.0	611.3	449.7	489.7
Urinary nitro-:gen (g)	18.7	18.8	17.6	17.4	10.9	12.5
METABOLIC MIXTURE						
Protein (g)	117	118	110	109	68	78
Fat (g)	124	128	24	30	153	198
Carbohydrate (g)	315	330	598	586	217	179
Water (g)	370	383	430	429	322	351
Calories (kcal)	2970	3080	3160	3170	2630	2940

Table 42.

The oxygen utilization and carbon dioxide output (both in ml./min.) and the R.Q.'s of rats in 4 groups with different carbohydrate intakes.

Group	Rat no.	O_2		CO_2		R.Q.		
		1	2	1	2	1	2	Mean
1	1	6.54	6.65	5.36	5.45	.82	.82	.82
	2	7.72	8.01	6.01	6.41	.78	.80	.79
	3	6.43	5.77	5.13	4.67	.80	.81	.81
2	4	8.10	8.93	6.81	7.59	.84	.85	.85
	5	8.80	10.34	6.84	8.38	.78	.81	.80
	6	7.80	8.08	6.52	7.26	.84	.90	.87
3	7	4.91	5.34	4.86	4.85	.99	.91	.95
	8	8.82	8.45	8.30	8.11	.94	.96	.95
	9	8.91	9.24	8.14	8.94	.91	.97	.94
4	10	9.54	9.63	9.32	9.72	.98	1.01	1.00
	11	6.62	5.75	7.20	6.32	1.09	1.10	1.10
	12	6.34	6.26	6.66	6.20	1.05	0.99	1.02
	13	5.42	5.26	5.34	5.30	0.99	1.01	1.00
	14	4.82	4.56	4.95	4.89	1.03	1.07	1.05
	15	6.81	6.93	6.79	7.02	1.00	1.01	1.01

Table 43.

The glycogen content (in g./100 g. tissue) found by analysis of the muscle, liver and skin of rats in 4 groups with different carbohydrate intakes.

GROUP	RAT NO.	GLYCOGEN IN:- LEG MUSCLE	ABDOMINAL MUSCLE	LIVER	SKIN
1	1.	.24	.35	1.4	.04
	2.	.26	.33	2.8	.06
	3.	.22	.34	2.0	.06
2.	4.	.27	.34	2.1	.02
	5.	.25	.31	0.9	.02
	6.	.25	.29	1.9	.05
3.	7.	.39	.56	3.6	.11
	8.	.44	.50	4.0	.16
	9.	.44	.49	4.8	.15
4.	10.	.41	.52	6.6	.06
	11.	.63	1.09	5.5	.33
	12.	.38	.52	5.0	.06
	13.	.71	.78	4.0	.11
	14.	.63	.80	5.6	.09
	15	.46	.64	8.4	.14

REFERENCES.

Adam, J.M., Best, T.W. & Edholm, O.G. (1961). J. Physiol. 156,38P.

Ashworth, N., Creedy, S., Hunt, J.N., Mahon, S. & New-:lands, P. (1962) Lancet ii, 685.

Atwater, W.O. & Benedict, F.G. (1903). Bull. U.S. Off. Exp. Stas. No. 136.

Atwater, W.O. & Bryant, A.P. (1900). Conn. (Storrs) Agric. Exp. Sta. 12th Annu. Rep., 1899. Quoted by Widdowson, E.M. (1960) in Spec. Rep. Ser.med. Res. Coun., Lond. No. 297.

Behnke, A.R., Osserman, E.F. & Welham, W.C. (1953). Arch. intern. Med. 91,585.

Benedict, F.G. & Carpenter, T.M. (1918). Publ. Carneg. Instn. No. 261.

Bergström, J., Findor, J. & Hultman, E. (1961). Scand. J. clin. Lab. Invest. 13,353.

Bondy, P.K., Sheldon, W.H. & Evans, L.D. (1949). J.clin. Invest. 28,216.

Bornstein, D. (1898). Berl. klin. Wschr. 35,791.

Bridge, E.M. (1937) Johns Hopk. Hosp. Bull. 61,349.

Brozek, J., Grande, F., Taylor, H.L., Anderson, J.T., Buskirk, E.R. & Keys, A. (1957) J. appl. Physiol.10,412.

Burghard, E. & Paffrath, H. (1927). Z.f. Kinderh. 37, 492. Quoted by MacIntyre, D.S., Pedersen, S. & Maddock, W.G. (1941) Surgery 10,716.

Cathcart, E.P. & Cuthbertson, D.P. (1931). J. Physiol. 72,349.

Cathcart, E.P. & Markowitz, J. (1927). J. Physiol. 63, 309.

Cohn, C. & Joseph, D. (1960). Amer. J. clin. Nutr. 8, 682.

Cori, G. T. (1954). Harvey Lect. 48,145.

Cori, C. F. & Cori, G. T. (1928). J. biol. Chem. 76, 755.

Cramer, A. (1888). Z. Biol. 24,67.

Cuthbertson, D.P., McCutcheon, A. & Munro, H.N. (1937). Biochem. J. 31,681

Cuthbertson, D.P., McGirr, J.L. & Munro, H.N. (1937). Biochem. J. 31,2293.

Cuthbertson, D.P. & Munro. H.N. (1937). Biochem. J. 31, 694.

Dahlström, H. & Wahlund, H. (1949). Scand. J. clin. Lab. Invest. 1,86.

Dapper, M. (1902) Ing. Diss. Marburg. Quoted by Magnus - Levy. A. (1907) in Metabolism and Practical Medicine, vol. 1, p315. Ed. by von Noorden, C. London: W. Heinemann.

Darke, S.J. (1960). Brit. J. Nutr. 14, 115.

Dewar, A.D. & Newton, W.H. (1948). Brit. J. Nutr. 2, 142.

Dole, V.P. (1956) J. clin. Invest. 35,150.

Dreyfus, J., Schapira, G., Schapira, F.& Demos, J. (1956) Clin. chim. Acta. 1, 434.

Durnin, J.V.G.A. (1961). Proc. Nutr. Soc. 20, 52.

Ellis, N.R. (1933). Tech, Bull. U.S. Dept. Agric. No. 368.

Entenman, C., Goldwater, W.H., Ayres, N.S. & Behnke, A.R. (1958). J. appl. Physiol. 13,129.

Fryer, J.H. (1958). Amer. J. clin. Nutr. 6, 354.

Furnass, B. (1960). J. Physiol. 150, 11P.

Garry, R.C., Passmore, R., Warnock, G.M. & Durnin, J.V. G.A. (1955). Spec. Rep. Ser. med. Res. Coun., Lond. No. 289.

Good, C.A., Kramer, H. & Somogyi, M. (1933). J. biol. Chem. 100,485.

Gulick, A. (1922). Amer. J. Physiol. 60,371.

Haex, A.J.C. (1944). Acta. med. scand. 118,531.

Hauk, R., Illingworth, B., Brown, D.H. & Cori, C.F. (1959). Biochim. biophys. Acta. 33, 556.

Hawley, E.E., Johnson, C.W. & Murlin, J.R. (1933). J. Nutr. 6,523.

Higgins, H.L. (1916). Amer. J. Physiol. 41,258.

Hildes, J.A., Sherlock, S. & Walshe, V. (1949). Clin. Sci. 7, 287.

Hines, H.M. & Knowlton, G.C. (1935) Amer. J. Physiol. 111,243.

Hollifield, G. & Parson, W. (1962). J. clin. Invest. 41, 250.

Holmes, E. G. & Trowell, H.C. (1948). Lancet i, 395.

Hug, G. (1961). Biochim. biophys. Acta. 47, 271.

Huggett, A. & Nixon, D.A. (1957). Lancet ii, 368.

Illingworth, B. (1961). Amer, J. clin. Nutr. 9, 683.

Illingworth, B. & Cori, G.T. (1952). J. biol. Chem. 199, 653.

Jacobs, S. (1959). Nature, Lond. 183, 262.

Johnson, R.E., Sargent, F.II. & Passmore, R. (1958). Quart. J. exp. Physiol. 43, 339.

Johnston, M.W. & Newburgh, L.H. (1942). J. clin. Invest. 21, 357.

Kayser, C. (1961). The Physiology of Natural Hibernation London: Pergamon Press.

Kekwick, A., Pawan, G.L.S. & Chalmers, T.M. (1959). Lancet ii, 1157.

Keys, A., Anderson, J.T. & Brozek, J. (1955). Metabolism 4, 427.

Keys, A. & Brozek, J. (1953). Physiol. Rev. 33, 245.

Krug, H. (1894). von Noorden's Beiträge 2, 83. Quoted by Luethje, H. (1902). Z. klin. Med. 44,22.

Lawes, J.B. & Gilbert, J.H. (1853). Rep. Brit. Ass. 22, 323.

Lloyd, B. B. (1958). J. Physiol. 143, 5P.

Longenecker, H.E. (1939). J. biol. Chem. 129,13.

Luethje, H. (1902). Z. klin. Med. 44,22.

MacIntyre, D.S., Pedersen, S. & Maddock, W.G. (1941) Surgery 10, 716.

Magnus - Levy, A. (1907). In Metabolism and Practial Medicine, vol.1, p. 185. Ed. by von Noorden, C. London: W. Heinemann.

Mommaerts, W.F., Illingworth, B., Pearson, C.M., Guillory, R. J. & Seraydarian, K. (1959). Proc. nat. Acad. Sci., Wash. 45, 791.

Newburgh, L. H., Johnston, M.W., Lashmet, F.H. & Sheldon, J.M. (1937). J. Nutr. 13, 203.

Newburgh, L.H. Wiley, F.H. & Lashmet, F.H. (1931). J. clin. Invest. 10,703.

Odier, J. & Mach, R.S. (1949). Praxis 38, 384. Quoted by McCance, R. A. & Widdowson, E.M. (1951). Proc. roy. Soc. B. 138,115.

Passmore, R. (1952). Unpublished.

Passmore, R. (1961). Nutr. Dieta. 3,1.

Passmore, R., Meiklejohn, A.P., Dewar, A.D. & Thow, R.K. (1955). Brit. J. Nutr. 9,20.

Passmore, R., Meiklejohn, A.P., Dewar, A.D. & Thow, R.K. (1955). Brit. J. Nutr. 9.27.

Passmore, R. & Swindells, Y.E. (1962). Biochem. J. 84, 117 P.

Pembrey, M.S. (1901-2). J. Physiol. 27, 407.

Peters, J.P. & Van Slyke, D.D. (1932). Quantitative Clinical Chemistry. Methods. p.626. London: Ballière, Tindall & Cox.

Pettenkofer, M. & Voit, C. (1866) Z. Biol. 2, 459.

Polglase, W.J. Smith, E.L. & Tyler, F.H. (1952) J. biol. Chem. 199,97.

Popper, H. & Wozasek, O. (1931). Virchows Arch. 279,819.

Popper, H. & Wozasek, O. (1931). Z. ges. exp. Med. 77, 414.

Robertson, J.D. & Reid, D.D. (1952). Lancet i, 940.

Shelley, H.J. (1961). Brit. med. Bull. 17,137.

Soderstrom, G.F. & Du Bois, E.F. (1917). Arch. intern. Med. 19,931.

Sokal, J.E. & Gerszi, K.E. (1959). J. Lab. clin. Med. 53. 876.

Sokal, J.E. & Sarcione, E.J. (1959). Amer. J. Physiol. 196, 1253.

Soskin, S. (1943). Arch. intern. Med. 71,219.

Soskin, S. & Levine, R. (1952). Carbohydrate Meta-:bolism. Correlation of Physiological, Biochemical and Clinical Aspects. Chicago: Univ. Chicago Press.

Strong, J.A., Passmore, R. & Ritchie, F.J. (1958). Brit. J. Nutr. 12, 105.

Taggart, N. (1962). Brit. J. Nutr. 16, 223.

Talbott, J.H., Coombs, F.S., Consolazio, W.V. & Pecora, L.J. (1938). Amer. J. Physiol. 124, 246.

Tepperman, J., Brobeck, J.R. & Long, C.N.H. (1943). Yale J. Biol. Med. 15,855.

Thin, C. & Robertson, A. (1952). Biochem. J. 51,218.

Trapp, H. (1850). Beiträge zur Kentniss der Veränderungen welche der Urin in Krankheiten erleidet. Thesis, Giessen. Quoted by Passmore, R., Strong, J.A. & Ritchie, F.J. (1959). Brit. J. Nutr. 13, 17.

Trevelyan, W.E. & Harrison, J.S. (1952) Biochem. J. 50, 298.

Voit, C. (1869) Z. Biol. 5, 431.

Werthessen, N.T. (1937). Amer. J. Physiol. 120, 458.

Wiley, F.H. & Newburgh, L.H. (1931). J. clin. Invest. 10, 723.

Wiley, F.H. & Newburgh, L.H. (1931). J. clin. Invest. 10, 733.

Wilson, H.E.C. (1931). J. Physiol. 72, 327.

Yater, W.M., Osterberg, A.E. & Hefke, H.W. (1930). Arch. intern. Med. 45,760.

Young, N.F., Abels, J.D. & Homburger, F. (1948). J. clin. Invest. 27, 760.

Zuntz. N. (1897). Pflüg. Arch. ges. Physiol. 68,191.

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